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THE COLLEGE OF AGRICULTURE

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A Study on the Marine Aerobic Cellulose-decomposing Bacteria

By

HAJIME KADOTA

KYOTO

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A Study on the Marine Aerobic Cellulose-decomposing Bacteria

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HAJIME KADOTA

With 36 Tables, 5 Text-figures and 23 Plates

(Received Dec. 20, 1955)

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Introduction

Although fishing nets made of synthetic fibers, such as nylon, vinylon, and salan have lately come into use, the greater part of the fishing gear now employed in our country is still made of natural cellulosic materials, *viz.* cotton, hemp, ramie, etc. Annual consumption of various textiles for fishing gear in Japan in 1953 was as follows.[1)]

Cotton	20,300,000 lbs
Ramie	600,000 lbs
Hemp	40,100,000 lbs
Nylon	1,290,000 lbs
Vinylon	700,000 lbs
Salan	530,000 lbs

Since natural fibers such as cotton, ramie, and hemp are produced only in small quantities in Japan, she must import them from foreign countries at great cost. In 1949 about ¥ 10,000,000,000 worth of these materials were imported.[2)]

Fishing gear made of such natural fibers depreciate rapidly and cannot be used over a long period.

It is estimated that about three quarters of the fishing nets are replaced annually in Japan.[3)]

It is thought that a large part of this need for replacement is a consequence of microbiological deterioration. According to ROBERTSON[4)], BARGHOORN[5)], CONN[3)], and

CARTER[7] the durability of such fishing gear was primarily affected by the activities of cellulose-decomposing microorganisms inhabiting the sea.

Cellulose-decomposing microorganisms, as well as those which attack lignin, are also instrumental in the destruction of timbers, wooden pilings and other cellulose containing structures exposed to the sea. Such microorganisms may often work symbiotically with shipworms or other wood borers.[8]

From the above mentioned facts, it is obvious that the damage to fishing gear through microbiological activity is a very serious problem in the fishing industry, especially in Japan.

The microbiological depreciation of fishing gear, however, has not attracted the serious attention of the microbiologist up to the present, and accordingly very little is known about the mechanism involved in such phenomenon.

Such being the case, the present work was undertaken with the aim of providing fundamental data from which future research may be directed toward the establishment of a complete method to prevent microbiological deterioration of fishing gear exposed to the sea.

In order to elucidate the mechanism of the microbial decomposition of fishing gear, it is essential to isolate pure cultures of such microorganisms and to study their distribution in the sea, their physiological characteristics, and their action upon certain purified cellulosic materials.

The following investigation, therefore, is mainly concerned with the ecology, taxonomy, physiology, and biochemistry of the cellulose-decomposing bacteria which may primarily be responsible for the deterioration of cellulosic fishing gear exposed to the sea.

Historical

It was early recognized that microorganisms were active in the deterioration processes of fishing nets exposed to the sea.

NISHIMURA and YAMAZOE[9], and KAWAI[10] observed that fishing nets made of cotton and hemp rapidly deteriorated in raw sea water, but they never deteriorated in sea water which had previously been sterilized. Their data, as presented in Table 1, show that the deterioration of such cellulosic fishing cords immersed in raw sea water was caused by the microorganisms which inhabited the sea.

From the cotton cords which had rotted by prolonged immersion in sea water NISHIMURA and YAMAZOE[9] isolated several crude cultures of microorganisms which were probably responsible for the deterioration, but they did not pursue the study further.

ATKINS[11),12] observed that cotton fishing lines rotted completely in 4 to 8 weeks in aquarium tanks containing sea water, and DOREE[13] found them to last 3 to 5 weeks

Table 1. Decrease in tensile strength of cotton and hemp cords during immersion in raw and sterilized sea water at room temperature. (From KAWAI[10])

Immersion time in days	Wet tensile strength of cotton cords in lbs		Wet tensile strength of hemp cords in lbs	
	In sterilized sea water	In raw sea water	In sterilized sea water	In raw sea water
0	28.38	28.38	25.83	25.83
28	28.83	14.40	25.85	16.80
43	28.63	7.38	26.40	15.00
58		12.06		12.90
73		18.40		
134		5.30		4.00
311	29.25			

when completely immersed in sea water.

BRANDT[14] found that the deterioration of nets exposed to brackish water was much more rapid during the summer months than during the winter. That this deterioration is influenced greatly by the temperature was clearly illustrated in his data presented in Fig. 1[14].

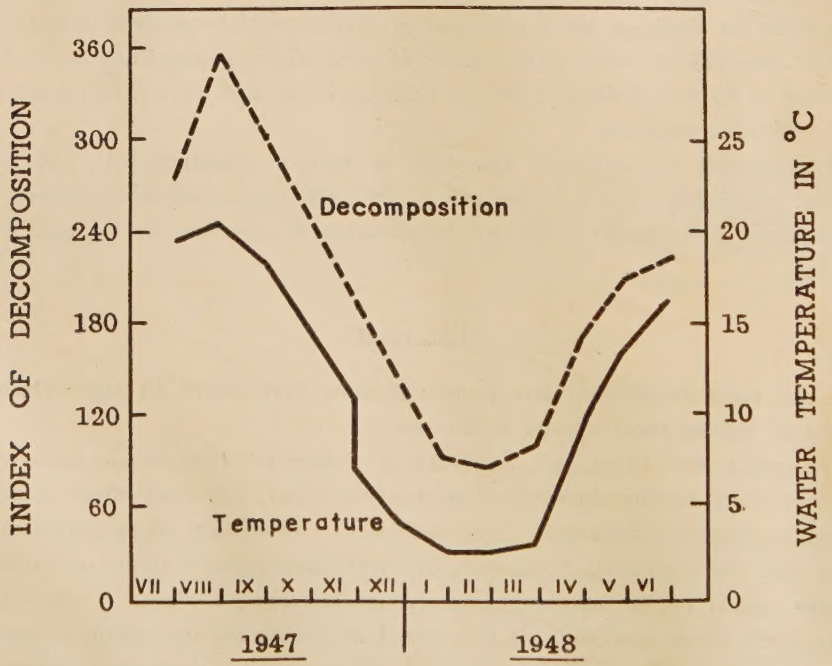


Fig. 1. Relation between rate of decomposition of cotton netting in ocean water and temperature during different times of the year. (From BRANDT[14])

DOREE[13] found that textile fibers were completely rotted after periods of from three to five weeks' immersion in the sea, and that the fabrics had become coated

with a bacterial or diatomaceous film within a week of immersion. He showed that the deterioration was caused by microorganisms and not by abiogenic oxidation, light, or salts.

KOTANI[15] reported that several cultures of marine bacteria which were isolated from the sea, decomposed cotton fishing nets submerged in the inorganic nutrient media. His data are presented in Table 2.

Table 2. Tensile strength of cotton cords after 21 and 38 days' immersion in inorganic nutrient media inoculated with marine bacteria. (From KOTANI[15])

Culture Number	Tensile strength of cotton cords	
	After 21 days' immersion at room temp.	After 38 days' immersion at 27°C.
Control (uninoculated)	13.9 lbs	13.3
A	7.9	3.0
D	11.9	10.5
E	12.4	10.4
F	11.6	5.4

The deterioration of fish nets in Lake Erie was found by ROBERTSON and WRIGHT[16], to be due to cellulose-digesting bacteria. Both linen and cotton lines, seines, and nets were attacked.

Manila ropes and cotton nets were found by ATKINS and WARREN[17] to be destroyed after 14 months' alternate wetting in sea water and drying, as in ordinary use. They found that preliminary treatment of the fibers with copper naphthenate prolonged the useful life of the rope by 40 per cent.

The occurrence of fungi, on the North Atlantic Coast, which infect cordage fibers, including hemp, jute, and sisal, causing deterioration under marine conditions was reported by BARGHOORN[18].

BARGHOORN and LINDER[19] investigated the physiology of several kinds of such fungi. Marine fungi which attack lignin and cellulose were believed by BARGHOORN and LINDER[19] to be partly responsible for the deterioration of hemp, jute, and sisal cordage as well as pilings and other wooden structures in the sea.

ISSATCHENKO[20] isolated from the mud of the salt lake Ssaky in Crimea two aerobic bacteria, one of which decomposed cellulose without forming a pigment (*Bact. cellulosae album*), while the other colored the paper yellow (*Bac. cellulosae flavum*).

From the deep water of Lake Tamubukan (Kaukasus) WALEDINSKY and SSWESCHNIKOFF[21] obtained crude cultures of aerobic cellulose-decomposing bacteria.

RUBENTSCHIK[22] isolated from the salt limans in Southern Russia an actinomyces (*Actinomyces melanogenes*) capable of slow decomposition of paper. Later he[23] found that anaerobic cellulose digesters were also distributed in such aquatic environments. The decomposition of cellulose by these organisms was unimpaired by a

salt concentration as high as 15 per cent NaCl.

In 1929 VAN DER LEK[24] demonstrated the occurrence in the sea of the bacterium, *Bacterium agar-liquefaciens*, which was capable of attacking cellulose and agar.

WAKSMAN, CAREY and REUSZER[25] found in the Gulf of Maine and George's Bank that cellulose-decomposing bacteria were generally present in sea water, and particularly abundant in bottom deposits and diatom tows. They[25] observed that species of *Cytophaga*, *Cellvibrio* and *Cellfalcicula* were present in the sea. Some of these bacteria were found to be capable of attacking both cellulose and agar, while others decomposed cellulose alone. Some of the bacteria were also able to decompose a number of other polysaccharides, as well as various mono- and disaccharides. Most of these cellulose-decomposing bacteria were aerobic. In crude cultures, the cellulose-decomposing bacteria were invariably accompanied by numerous protozoans, including flagellates, ciliates, and amoebae.

WAKSMAN et al.[25], however, made no attempt to describe and c'assify such bacteria.

BAVENDAMM[26] found aerobic cellulose digesters in all marine mud samples, and anaerobic cellulose digesters in several of the samples which he examined.

Using the minimum dilution method, ZOBELL[27] demonstrated 1000 cellulose digesters per gram of marine mud.

In "Marine Microbiology" ZOBELL[28] stated that marine microorganisms which were endowed with the ability to utilize cellulose might be demonstrated in most 10- to 100-ml samples of sea water and in nearly all one gram samples of bottom deposits.

From marine materials, STANIER[29] isolated and described *Vibrio fuscus*, *Pseudomonas iridescens*, *Cytophaga krzemieniewskae*, and *Cytophaga diffluens*, all being new species which digested cellulose and agar. According to STANIER[29] *Vibrio granii* and *Pseudomonas droebachense*, two species previously named by LUNDESTED[30], digested both agar and cellulose.

Using fresh water media, KOTANI[15] obtained from sea water several cultures of aerobic cellulose-decomposing bacteria which he described but failed to name.

In a research for marine agar-digesting bacteria of the South Atlantic Coast of North America, HUMM[31] isolated and described six species, which digested cellulose and agar, *viz.*, *Vibrio agar-liquefaciens*, *Pseudomonas droebachense*, *Pseudomonas iridescens*, *Vibrio notus*, *Vibrio stanierii*, *Pseudomonas elongata*.

HIDAKA[8] isolated a cellulose-decomposing bacterium from the intestinal canal of *Toredo*. This bacterium rapidly decomposed cellulose in sea water media.

More than 20 strains of marine bacteria were reported by the above mentioned workers as cellulose decomposers. The greater part of these organisms, however, were not isolated in pure culture and were only insufficiently studied.

In a previous study concerning cellulose-decomposing bacteria found in Maizuru Bay, the present author[32-36] isolated and described four new species and two new

varieties of marine aerobic bacteria, *viz.*, *Vibrio purpureus*, *Vibrio purpureus* var. *albus*, *Vibrio marinoflavescens*, *Cytophaga haloflava*, *Cytophaga haloflava* var. *nonreductans*, and *Cytophaga rosea*, all being able to decompose cellulose in sea water media.

A list of previously described species of cellulose-decomposing bacteria of marine origin is as stated in appendix.

Experiments

I. Isolation and enumeration of marine cellulose-decomposing bacteria.

The following three methods were employed for the isolation of cellulose-decomposing bacteria from sea water, marine mud, and other marine materials.

1. Enrichment culture method

In the case of the isolation from sea water, where only a few cellulose-decomposing bacteria were present, the selective enrichment culture method was usually used as a preliminary process for the isolation.

In order to set up the medium and the incubating temperature suitable for the enrichment culture of cellulose-decomposing bacteria, various kinds of media were examined at different temperatures by the successive dilution method.

The successive dilution method consisted of diluting the sea water or marine mud by powers of 10, inoculating equal volumes of the diluted materials into the liquid media to be examined, and incubating the inoculated media at different temperatures.

The reciprocal of the highest dilution which yielded a positive result was regarded as the most probable number of cellulose-decomposing bacteria detected in the sample. The medium which consistently gave the highest number of cellulose-decomposing bacteria from the identical sample was regarded as the fittest medium for the enrichment culture of marine cellulose-decomposing bacteria. The fittest temperature for incubation was selected in the same way.

The compositions of the enrichment culture media tested were as follows:

Medium 1 (Hutchinson & Clayton's medium[37])

K_2HPO_4	1.0 g
$CaCl_2$	0.1
$MgSO_4 \cdot 7H_2O$	0.3
$FeCl_3$	0.01
$NaNO_3$	2.5
A strip of filter paper	
Water	1000.0 ml
pH	7.2

Medium 2 (Modified Hutchinson & Clayton's medium[37])

K_2HPO_4	1.0g
$CaCl_2$	0.1
$MgSO_4 \cdot 7H_2O$	0.3
$FeCl_3$	0.01
$NaNO_3$	2.5

	A strip of filter paper	
	Filtered sea water	1000.0 ml
	pH	7.2
Medium 3	(OMELIANSKY's medium 1[38])	
	Peptone	1.0 g
	K_2HPO_4	1.0
	$MgSO_4 \cdot 7H_2O$	0.5
	$CaCO_3$	2.0
	NaCl	0.01
	A strip of filter paper	
	Distilled water	1000.0 ml
	pH	7.2
Medium 4	(OMELIANSKY's medium 2[38])	
	$(NH_4)_2SO_4$	1.0 g
	K_2HPO_4	1.0
	$MgSO_4 \cdot 7H_2O$	0.5
	$CaCO_3$	2.0
	NaCl	0.01
	A strip of filter paper	
	Distilled water	1000.0 ml
	pH	7.2
Medium 5	(Modified OMELIANSKY's medium 1[38])	
	Peptone	1.0 g
	K_2HPO_4	1.0
	$MgSO_4 \cdot 7H_2O$	0.5
	A strip of filter paper	
	Filtered sea water	1000.0 ml
	pH	7.2
Medium 6	(Modified OMELIANSKY's medium 2[38])	
	$(NH_4)_2SO_4$	1.0 g
	K_2HPO_4	1.0
	$MgSO_4 \cdot 7H_2O$	0.5
	A strip of filter paper	
	Filtered sea water	1000.0 ml
	pH	7.2
Medium 7	(Modified KADOTA's medium[39])	
	$NaNO_3$	0.5 g
	K_2HPO_4	1.0
	$MgSO_4 \cdot 7H_2O$	0.5
	$FeSO_4 \cdot 7H_2O$	0.01
	A strip of filter paper	
	Filtered sea water	1000.0 ml
	pH	7.2
Medium 8*	(Modified OMELIANSKY's medium 2 for anaerobic bacteria I [38])	
	$(NH_4)_2SO_4$	1.0 g
	K_2HPO_4	1.0
	$MgSO_4 \cdot 7H_2O$	0.5
	L-Ascorbic acid	100.0 mg
	A strip of filter paper	

Filtered sea water	1000.0 ml
pH	7.2

Medium 9* (Modified OMELIANSKY's medium 2 for anaerobic bacteria II[38])

$(NH_4)_2SO_4$	1.0 g
K_2HPO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.5
M/10 Na_2S	15.0 ml
A strip of filter paper	
Filtered sea water	1000.0 ml
pH	7.2

Medium 10* (Modified KADOTA's medium for anaerobic bacteria I[36])

$NaNO_3$	0.5 g
K_2HPO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.5
$FeSO_4 \cdot 7H_2O$	0.01
L-Ascorbic acid	100.0 mg
A strip of filter paper	
Filtered sea water	1000.0 ml
pH	7.2

Medium 11* (Modified KADOTA's medium for anaerobic bacteria II[36])

$NaNO_3$	0.5 g
K_2HPO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.5
$FeSO_4 \cdot 7H_2O$	0.01
M/10 Na_2S	15.0 ml
A strip of filter paper	
Filtered sea water	1000.0 ml
pH	7.2

In the cases of the media 1, 2, 3, 4, 5, 6, and 7, all of the soluble components dissolved in fresh or sea water were directly poured into test tubes (about one-third full). A strip of filter paper was placed in each of the above mentioned tubes in such a way as to protrude above the surface of the liquid. The media thus prepared were sterilized by autoclaving.

In the cases of the remaining media, L-ascorbic acid or Na_2S separately sterilized by passing through SEITZ filter was carefully mixed with the previously autoclaved solutions containing other soluble components, and dispensed into test tubes (about a half full). A strip of filter paper was added to each of the above tubes so as to be entirely immersed in the liquid.

All the media thus prepared were inoculated with successively diluted water or mud samples collected from Maizuru Bay and incubated at 20°, 25°, 30°, or 35°C.

Effects of the composition of the medium and the incubating temperature upon the development of marine cellulose-decomposing bacteria are summarized in Table 3 which shows the results obtained by this method.

* Two ml portions of sterilized liquid paraffin were added to each tube after sterilization.

Table 3. Approximate numbers of cellulose-decomposing bacteria demonstrated by the successive dilution method using various media from 1 ml of sea water samples or 1 g of marine mud samples at different temperatures.

Medium No. \ Sample / Temp. °C / Time in days	1 ml of sea water collected at 0 m depth in Maizuru Bay								1 ml of sea water collected at 2 m depth in Maizuru Bay								1 g of marine mud collected in Maizuru Bay							
	20		25		30		35		20		25		30		35		20		25		30		35	
	6	12	6	12	6	12	6	12	6	12	6	12	6	12	6	12	6	12	6	12	6	12	6	12
1	0	1	1	1	1	1	1	1	0	1	0	0	1	1	0	0	0	0	0	1	0	1	0	0
2	1	10	10	100	10	10	1	10	1	10	10	10	10	10	0	0	1	100	100	100	10	10	1	1
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1*	1	1*
4	0	1	1	1	1	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	1	1*	0	0
5	1	10	1	1	1	1	10	10	0	1	1	1	1	1	0	0	0	0	0	1	1	1	0	0
6	1	10	10	10	10	10	1	10	1	10	10	10	10	10	1	1	0	100	100	100	10	100	10	10
7	1	10	100	100	10	10	1	10	1	10	10	10	10	10	1	1	1	100	100	1000	10	10	10	10
8	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	10	10	10*	10	10*	1	1*
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1*	10	10*	0	0
10	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10*	1	1*
11	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	1*	1	0

* A small number of anaerobic forms were detected.

As shown in Table 3, cellulose-decomposing bacteria were found to exist generally, as normal flora, in sea water and bottom deposits in Maizuru Bay.

Most of the cellulose-decomposing bacteria developed were aerobic, but a few of them were anaerobic forms which were found only in marine mud. These results accord with those observed by WAKSMAN et al.[25] in the Gulf of Maine and George's Bank.

With the exception of stagnating water, sea water in the euphotic zone generally contains oxygen in high concentration. The oxygen content of sea water in such areas sometimes exceeds the saturation value[40]. The oxidation-reduction potential in sea water has been considered by COOPER[41] to be associated with the partial pressure of oxygen in the water. Under conditions of high oxygen pressure, such as in the euphotic zone, the oxidation-reduction potential of sea water may generally be suitable for the growth of aerobic bacteria, but it may be too high for the exsistence of anaerobic bacteria.

From the above mentioned, it is thought that the microbial deterioration of cellulosic fishing nets immersed in sea water may be primarily caused by the activities of aerobic cellulose-decomposing bacteria.

Maximum count was obtained on medium 7 incubated at 20°C and 25°C. At the case of 20°C, however, the length of time required for reaching the maximum count was prolonged, since the development of the bacteria was considerably slower. These results show that medium 7, among the media examined, was the fittest for the enrichment culture, and that the temperature of 25°C was the fittest for incubation.

In the following experiments, therefore, medium 7 was generally employed under aerobic condition at 25°C for the purposes of the selective enrichment and the enumeration of cellulose-decomposing bacteria. Medium 7 differs very little in composition from the medium, recommended previously by the author[39], for the cultivation of marine agar-digesting bacteria.

As shown in Table 3, the count of cellulose-decomposing bacteria obtained was invariably higher in the media prepared with sea water than in the media prepared with fresh water. This probably is as it should be as the bacteria originated from the sea. Similar results have been recognized, since early times, as to other marine heterotrophs.[28][42][43][44] KORINEK[45] reported that marine bacteria could be geneally distinguished from non-marine forms on a basis of their salt tolerance.

2. The cellulose agar plate method.

WAKSMAN et al.[25] have reported obtaining a pure culture of marine cellulose-decomposing bacteria by repeating the enrichment culture method as stated above, but the author found it impossible to isolate these bacteria in pure by such a method only.

In the present experiment, therefore, for obtaining pure cultures of cellulose-decomposing bacteria, cellulose agar plate methods were used.

The cellulose agar medium employed for this procedure was made up to contain the following composition :

$NaNO_3$	0.5 g
K_2HPO_4	1.0
$MgSO_4 \cdot 7H_2O$	0.5
$FeSO_4 \cdot 7H_2O$	0.01
Precipitated cellulose*	12.0
Washed agar	15.0
Filtered sea water	1000.0 ml
pH adjusted to	7.2

Procedure of the plating was as follows :

After about 1 ml of the appropriately diluted inoculants (sea water, marine mud, other marine materials or selective enrichment cultures) and about 8 ml of sterilized sea water** were placed in sterilized petridishes, about 9 ml of the melted cellulose-agar medium was poured in the dishes and thoroughly mixed with inoculants. After the agar had been solidified, the plate was incubated at 25°C.

** Sterilized sea water was added for the following purposes :

a) To avoid the harmful effect of high plating temperatures, because marine cellulose-decomposing bacteria are generally sensitive to high plating temperatures and may sometimes be injured by exposure to the usual plating temperature of agar (40–45°C) for ten minutes.[46]

b) To promote the growth of cellulose-decomposing bacteria such as cytophagas, because cytophagas grow well on weak agar gels but hardly on rigid agar gels,[47)–49)]—on weak agar gels these organisms, instead of being confined to a small area where the food supply is rapidly used up, can creep outwards in all direction through the agar, but they cannot on rigid agar gels.

c) To prevent the plate drying up during incubation, after plating, because in general, marine cellulose-decomposing bacteria grow more slowly than the bacteria of other origin,—more than 10 days' incubation is often required for the full development of marine cellulose-decomposing bacteria on cellulose agar plates.

In the case of the isolation of the cellulose-decomposing bacteria belonging to *Eubacteriales*, the crude cultures preliminarily enriched by the method mentioned above were generally used as inoculants. In the case of *Myxobacterials*, however, samples of sea water, marine mud or other marine materials were directly inoculated to cellulose agar plates using various dilutions ***.

*** As will be shown later, most of the marine materials contain fewer myxobacteria than true bacteria, and true bacteria usually develop more rapidly than myxobacteria in liquid enrichment culture media. Therefore, preliminary enrichment treatment using a liquid medium is somewhat disad-

* Precipitated cellulose was prepared by copper-ammonia method.

vantageous for the isolation of myxobacteria.

Owing to its accumulative nature, such a enrichment culture tends to yield only such bacteria as *Vibrio* which grow rapidly and exclusively.

After 7 to 14 days' incubation several types of cellulose-decomposer usually developed on the plate. The bacteria were picked up from each of the colonies which developed on the plates inoculated with appropriately diluted samples and transferred to a new enrichment culture medium, and the preceding process was repeated until the purity of the culture was confirmed by microscopic and naked-eye observations.

The colonies of cytophagas appeared on such plates after 7 to 10 days' incubation at 25°C in the form of small yellow, orange, or pink mucoid spots. After this time these spots increased gradually in size and became translucent due to the complete dissolution of the fiber structure within them. This translucency was highly characteristic of a cytophaga colony.

3. Natural enrichment culture method.

For the purpose of isolation of cytophagas from the sea, the separative natural enrichment technique in which a piece of cotton fishing net was immersed in sea water was markedly superior.

The enrichment of marine cellulose-decomposing bacteria belonging to genus *Cytophaga* was hardly possible by means of the enrichment culture method, as above described, owing to their scarcity in the sea and to their slow development in the medium.

The present author has previously ascertained that these bacteria have a characteristic by which they are able to grow better or possibly only on a submerged surface, such as fishing nets, than in a free floating condition. For the enrichment of these bacteria, therefore, this characteristic was utilized, *viz.*, a piece of cotton fishing net was immersed in the sea for about 10 days before sampling.

Since these bacteria could be easily enriched on the cotton nets under the natural environment by means of this method, the cotton net was used as the sample for the isolation of these bacteria, *viz.*, it was inoculated to the cellulose-agar plates immediately after diluted to various degrees.

II. Classification and descriptions of species.

By use of the above mentioned methods, 252 pure cultures of marine cellulose-decomposing bacteria were isolated. All of the cultures isolated were strict aerobes.

These isolated cultures were classified into the following 3 genera–18 species and 2 varieties after detailed taxonomical studies.

Of these, 14 species were proposed as new species.

Order *Eubacteriales* Buchanan.

Family *Pseudomonadaceae* Winslow et al.

Tribe *Pseudomonadeae* Kluyver and Van Niel.

Genus *Pseudomonas* MIGULA.

1. *Pseudomonas marinocellulosae* nov. sp.

Tribe *Spirilleae* KLUYVER and VAN NIEL.

Genus *Vibrio* MÜLLER.

2. *Vibrio aquamarinus* nov. sp.
3. *Vibrio purpureus* KADOTA.
4. *Vibrio purpureus* var. *albus* KADOTA.
5. *Vibrio gilvus* nov. sp.
6. *Vibrio albogilvus* nov. sp.
7. *Vibrio macerans* nov. sp.
8. *Vibrio marinoliquefaciens* nov. sp.
9. *Vibrio simplex* nov. sp.
10. *Vibrio ferrugineus* nov. sp.
11. *Vibrio brevis* nov. sp.
12. *Vibrio fulvus* nov. sp.
13. *Vibrio aurantiacus* nov. sp.
14. *Vibrio marinotypicus* nov. sp.
15. *Vibrio euryhalis* nov. sp.
16. *Vibrio marinoflavescens* KADOTA.
17. *Vibrio xanthus* nov. sp.

Order *Myxobacteriales* JAHN.

Family *Cytophagaceae* STANIER.

Genus *Cytophaga* WINOGRADSKY.

18. *Cytophaga haloflava* KADOTA.
19. *Cytophaga haloflava* var. *nonreductans* KADOTA.
20. *Cytophaga rosea* KADOTA.

1. Descriptions of species.

Pseudomonas marinocellulosae nov. sp.

(Pl. 1)

Etymology: Latin marinus, pertaining to the sea, and M. L. cellulosa, cellulose.

Morphology

Vegetative cells: Straight rigid rods, with rounded ends. Cells 0.35 microns broad by 2.0 to 3.0 microns long. Actively motile by means of a single polar flagellum. Occurring singly and rarely in pairs. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth becomes apparent after 3 to 4 days (at 25°C) as a brownish area on the paper at the air-water interface or a little above it. The growth area has a tendency to spread upwards, but there is never visible development below the water level. Pigment produced is non water-

soluble. The liquid becomes turbid and semi-opaque.

Cellulose is rapidly decomposed. The attacked filter paper becomes weak and macerated, but there is never complete lysis. On shaking, the attacked paper breaks up into particles and shreds of fibers.

On mineral agar plates (prepared with sea water) containing finely divided precipitated cellulose, ochre-colored, opaque to semi-opaque, glistening, thin, cirucular colonies appear after 3 to 4 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition apparently does not take place. Colonies are surrounded by narrow enzymatic zones of cellulose dissolution.

Broth: No growth.

Sea water broth: Heavy turbidity, usually without pellicle.

Glucose broth: No growth.

Sea water glucose broth: Heavy turbidity.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: Ochre colored, opaque to semi-opaque, glistening, thin, circular colonies develop. Agar is not decomposed.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: Ochre colored, opaque to semi-opaque, glistening, thin, circular colonies develop. Agar is not decomposed.

Glucose agar slant prepared with sea water: Ochre colored, glistening, opaque to semi-opaque, thin, echinulate.

Starch agar plate prepared with sea water: The same as glucose agar plate prepared with sea water.

Starch agar slant prepared with sea water: The same as glucose agar slant prepared with sea water.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Ochre-colored, semi-opaque, glistening, thin, circular colonies develop.

Glucose mineral agar slant prepared with sea water: Ochre-colored, glistening, semi-opaque, thin, fili-form.

Starch mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose

mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrate is reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed and in sea water nitrate broth.

NH_3 produced from peptone in sea water broth.

Indol not formed from peptone in sea water broth and in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in sea water broth and in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Xylose, glucose, fructose, galactose, cellobiose, maltose, trehalose, sucrose, cellulose, starch, glycerin, mannitol, and Na-alginate.

Not utilized: Arabinose, rhamnose, mannose, lactose, raffinose, inulin, agar, erythritol, inositol, dulcitol, salicin, Ca-lactate, and Ca-gluconate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, and peptone.

Not utilized: $NaNO_2$.

Salt concentration range for growth: No growth in the media prepared with sodium chloride solution having a salinity of 0–10%. Growth occurrs exclusively in media prepared with natural sea water.

pH range for growth: 6.0–9.0.

Optimum pH: 7.0–8.0.

Temperature range for growth: 15–30°C.

Optimum temperature: 25°C.

Thermal death point: 50°C. for 5 minutes.

Aerobic.

Source: Three cultures were isolated from sea water collected at Maizuru Bay.

Habitat: The sea.

Vibrio aquamarinus nov. sp.

(Pl. 2)

Etymology: Latin aqua, water, and marina, sea.

Morphology

Vegetative cells: Slightly curved slender rods with rounded ends. Cells 0.3 by 2.0 to 3.6 microns, usually occurring singly but sometimes in pairs. Cells sometimes straight in old cultures. Motile with a single polar flagellum. Gram negative. Stain uniformly with GIEMSA's stain. No spores formed.

Cultural and physiological characteristics.

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth becomes apparent after 2 days (at 25°C) as a reddish purple area on the paper at the air-water interface or a little above it. The liquid itself remains comparatively clear. Cellulose is rapidly decomposed. The attacked filter paper becomes weak and macerated, but there is never a complete lysis. On shaking, the attacked paper breaks up into particles and shreds of fibers. Appearance of the growth of this species on the paper closely resembles to that of Vibrio purpureus.

On mineral agar plates (prepared with sea water) containing finely divided precipitated cellulose, brownish red, opaque, glistening, thin, colonies appear in 2 to 3 days. Colonies become spreading with age. Margin of spreading colonies irregular. Colonies usually form deep depression in agar. Colonies are not surrounded by distinct enzymatic zones of cellulose dissolution; fibrolysis takes place only in the immediate neighborhood of organisms. Agar is rapidly decomposed, and ultimately liquefaction becomes almost complete.

Broth: No growth.

Sea water broth: No growth.

Glucose broth: No growth.

Sea water glucose broth: slightly turbid; thin reddish pellicle may develop.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: Brownish red, opaque, glistening, thin, circular colonies develop. After a few days, colonies become spreading. Margin of spreading colonies irregular. A diffusible brown to black pigment produced in the agar after prolonged incubation. Colonies markedly sunken in agar; agar rapidly digested and liquefied.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: Moderate growth. The same as sea water agar plate.

Starch agar plate prepared with sea water: Moderate growth. The same as sea water agar plate.

Mineral agar plate prepared with sea water: Brownish red, semi-opaque, glistening, thin, circular colonies develop. Colonies become spreading with age. Colonies sunken in agar; Agar rapidly liquefied.

Glucose mineral agar plate prepared with sea water: The same as the above.

Starch mineral agar plate prepared with sea water: The same as the above.

Cellobiose mineral agar plate prepared with sea water: The same as the above.

Potato: No growth.

Milk: No growth.

Nitrite not produced from nitrate in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Xylose, arabinose, glucose, galactose, mannose, cellobiose, maltose, lactose, sucrose, cellulose, starch, agar, Na-alginate, and salicin.

Not utilized: Rhamnose, fructose, trehalose, raffinose, inulin, glycerin, erythritol, mannitol, inositol, dulcitol, Ca-lactate, and Ca-gluconate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salt concentration range for growth: 1.0–3.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 6.0–9.0.

Optimum pH: 7.0.

Temperature range for growth: 20–35°C.

Optimum temperature: 25–30°C.

Thermal death point: 40°C for 40 minutes, 50°C for 5 minutes.

Aerobic.

Source: Sixteen cultures were isolated from sea water collected from Maizuru Bay.

Habitat: The sea.

Vibrio purpureus KADOTA.[32)39)]

(Pl. 3)

Morphology

Vegetative cells: Slightly curved slender rods, 0.3 to 0.4 by 1.8 to 3.5 microns with rounded ends, usually occurring singly but sometimes in pairs. Cells sometimes straight in old cultures. Actively motile by means of a single polar flagellum.

Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, brownish purple growth becomes apparent after 2 days (at 25°C) on the paper at the air-water interface or a little above it. The liquid itself remains comparatively clear. Cellulose is rapidly decomposed. The attacked filter paper becomes weak and macerated, but there is never a complete lysis. On shaking, the attacked paper at the air-water interface breaks up into particles and shreds of fibers.

On mineral agar plates (prepared with sea water) containing finely divided precipitated cellulose, reddish brown, opaque, thin, circular colonies appear in 2 to 3 days. Colonies become spreading with age. Margin of spreading colonies irregular. Colonies form deep depression in agar. Colonies are not surrounded by distinct enzymatic zones of cellulose dissolution; fibrolysis takes place only in the immediate neighborhood of organisms. Agar is rapidly decomposed, and ultimately liquefaction becomes almost complete.

Broth: No growth.

Sea water broth: No growth.

Glucose broth: No growth.

Sea water glucose broth: Slightly turbid; brownish purple pellicle may develop.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: Very small, circular, opaque, brownish colonies develop after prolonged incubation. Colonies are later slightly depressed.

Sea water agar plate: Brownish, opaque, glistening, thin, circular colonies develop. In a few days, colonies become spreading. Margin of spreading colonies irregular. A diffusible dark pigment produced in the agar after prolonged incubation. Colonies markedly sunken in agar; agar rapidly digested and liquefied.

Glucose agar plate: The same as agar plate.

Starch agar plate: The same as agar plate.

Glucose agar plate prepared with sea water: The same as sea water agar plate.

Starch agar plate prepared with sea water: The same as sea water agar plate.

Mineral agar plate prepared with sea water: Brownish red, semi-opaque, glistening, thin, circular colonies develop. Colonies become spreading with age. Colonies sunken in agar; agar rapidly liquefied.

Glucose mineral agar plate prepared with sea water: The same as the above.

Starch mineral agar plate prepared with sea water: The same as the above.

Cellobiose mineral agar plate prepared with sea water: The same as the above.

Potato: No growth.

Milk: No growth.

Nitrate rapidly reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Glucose, fructose, galactose, mannose, cellobiose, sucrose, cellulose, starch, agar, salicin, and Na-alginate.

Not utilized: Xylose, arabinose, rhamnose, maltose, lactose, trehalose, raffinose, inulin, glycerin, erythritol, mannitol, inositol, dulcitol, Ca-lactate, and Ca-gluconate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salt concentration range for growth: 0.5–0.3%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 6.0–9.0.

Opimum pH: 7.0.

Temperature range for growth: 15–35°C.

Optimum temperature: 25–30°C.

Thermal death point: 50°C for 5 minutes.

Aerobic.

Source: Thirty one cultures were isolated from sea water and other marine materials collected in Maizuru Bay.

Habitat: The sea.

Vibrio purpureus var. *albus* KADOTA.[32]

(Pl. 4)

Morphology

Vegetative cells: Slightly curved, or straight rods, with rounded ends, 0.35 to 0.45 by 2.0 to 4.0 microns, usually occurring singly but sometimes in pairs. Actively motile by means of a single polar flagellum. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics.

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea

water) with a strip of filter paper partly immersed, growth becomes apparent after 2 days (at 25°C) on the paper at the air-water interface. No pigment produced on the filter paper. The liquid becomes faintly turbid. Cellulose is rapidly decomposed. The attacked filter paper becomes weak and macerated, but there is never a complete lysis. On shaking, the attacked paper breaks up into particles and shreds of fibers.

On mineral agar plates (prepared with sea water) containing finely divided precipitated cellulose, brownish white, opaque, thin, circular to irregular colonies appear in 2 to 3 days. Colonies usually form deep depression in agar. Colonies are not surrounded by distinct enzymatic zones of cellulose dissolution; fibrolysis takes place only in the immediate neighborhood of organisms. Agar is rapidly decomposed, and ultimately liquefaction becomes almost complete.

Broth: No growth.

Sea water broth: No growth.

Glucose broth: No growth.

Sea water glucose broth: Somewhat turbid; thin whitish pellicle may develop.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: Punctiform, circular, opaque colonies develop after prolonged incubation.

Sea water agar plate: Milk-white, opaque, glistening, thin, circular to irregular colonies develop. Colonies markedly sunken in agar; agar rapidly digested and liquefied.

Glucose agar plate: The same as agar plate.

Starch agar plate: The same as agar plate.

Glucose agar plate prepared with sea water: The same as sea water agar plate.

Starch agar plate prepared with sea water: The same as sea water agar plate.

Mineral agar plate prepared with sea water: Milk-white, semi-opaque, glistening, thin, circular colonies develop. Colonies become spreading with age. Colonies sunken in agar: Agar rapidly liquefied.

Glucose mineral agar plate prepared with sea water: The same as the above.

Starch mineral agar plate prepared with sea water: The same as the above.

Cellobiose mineral agar plate prepared with sea water: The same as the above.

Potato: No growth.

Milk: No growth.

Nitrate rapidly reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with

filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Glucose, fructose, galactose, cellobiose, sucrose, cellulose, starch, agar, Na-alginate, and salicin.

Not utilized: Xylose, arabinose, rhamnose, mannose, trehalose, maltose, lactose, raffinose, inulin, glycerin, erythritol, mannitol, inositol, dulcitol, Ca-lactate, and Ca-gluconate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, and peptone.

Not utilized: Histidine and $NaNO_2$.

Salt concentration range for growth: 0.5–3.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 6.0–9.0.

Optimum pH: 7.0–8.0.

Temperature range for growth: 15–35°C.

Optimum temperature: 30°C.

Thermal death point: 50°C for 5 minutes.

Aerobic.

Source: Eighteen cultures were isolated from sea water and other marine materials collected from Maizuru Bay.

This organism bears some resemblance to *Vibrio agarliquefaciens* (GRAY and CHALMERS) BERGEY et al. and *Vibrio notus* HUMM, but it differs from them with respects to the size of cells, the production of H_2S from peptone and the utilization of carbohydrates.

Vibrio gilvus nov. sp.

(Pl. 5)

Etymology: Latin gilvus, pale yellow.

Morphology

Vegetative cells: Slightly curved slender rigid rods, with rounded ends, usually C-shaped, with occasional S-shaped form. Cells 0.25 to 0.3 microns broad by 3.0 to 5.0 microns long. Actively motile with a polar flagellum. Occurring singly and rarely in pairs.

Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics.

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea

water) with a strip of filter paper partly immersed, growth becomes apparent in 3 to 4 days (at 25°C) as a pale yellow area on the paper at the air-water interface or a little above it. The growth area has a tendency to spread upwards, but there is never visible development below water level. Pigment produced is non water-soluble. The liquid becomes slightly turbid and semi-opaque.

Cellulose is rapidly decomposed. The attacked filter paper becomes weak and macerated, but there is never complete lysis. On shaking, the attacked paper breaks up into particles and shreds of fibers.

On mineral agar plates (prepared with sea water) containing finely divided precipitated cellulose, pale yellow, opaque, glistening, thin, circular colonies appear after 3 to 4 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition apparently does not take place.

Colonies are not surrounded by enzymatic zones of cellulose dissolution; attack on the cellulose takes place only in the immediate neighborhood of organisms.

Broth: No growth.

Sea water broth: No growth.

Glucose broth: No growth.

Sea water glucose broth: Slightly turbid.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: No growth.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: Pale yellow, glistening, semi-opaque, flat, circular, small colonies develop. Agar is not decomposed.

Glucose agar slant prepared with sea water: Feeble growth, pale yellow, glistening, semi-opaque, flat, echinulate.

Starch agar plate prepared with sea water: The same as glucose agar plate prepared with sea water.

Starch agar slant prepared with sea water: Feeble growth becomes apparent exclusively around the condensed water. Pale yellow, glistening, semi-opaque, flat, echinulate.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Pale yellow, semi-opaque, glistening, thin, circular colonies develop.

Glucose mineral agar slant prepared with sea water: Growth becomes apparent exclusively around the condensed water. Pale yellow, glistening, semi-opaque, flat, filiform to echinulate.

Starch mineral agar plate prepared with sea water: The same as glucose mineral

agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrite not produced from nitrate in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Arabinose, glucose, galactose, mannose, cellobiose, maltose, lactose, sucrose, cellulose, starch, and salicin.

Not utilized: Xylose, rhamnose, fructose, trehalose, raffinose, inulin, agar, glycerin, erythritol, mannitol, inositol, Ca-lactate, Ca-gluconate, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, urea, and peptone.

Not utilized: $NaNO_2$, $(NH_4)_2SO_4$, NH_4Cl, aspartic acid and histidine.

Salt concentration range for growth: 1.0–3.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 7.0–9.0.

Optimum pH: 8.0.

Temperature range for growth: 25–35°C.

Optimum temperature: 30°C.

Thermal death point: 40°C for 40 minutes, 50°C for 5 minutes.

Aerobic.

Source: Thirteen cultures were isolated from sea water collected from Maizuru Bay.

Habitat: The sea.

Vibrio albogilvus nov. sp.

(Pl. 6)

Etymology: Latin albus, white, and gilvus, pale yellow.

Morphology

Vegetative cells : Slightly curved slender rigid rods, with rounded ends. Cells 0.30 to 0.35 microns broad by 2.2 to 3.8 microns long. Actively motile by means of a polar flagellum. Occurring singly and rarely in pairs. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics.

Growth on cellulose : In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth becomes apparent after 2 to 3 days (at 25°C) as a pale cream yellow area on the paper at the air-water interface. Pigment produced is not water soluble. The liquid becomes slightly turbid and semi-opaque.

Cellulose is rapidly decomposed. The attacked filter paper becomes weak and macerated, but there is never complete lysis. On shaking, the attacked paper breaks up into particles and shreds of fibers.

On mineral agar plates (prepared with sea water) containing finely divided precipitated cellulose, pale cream yellow, semi-opaque, glistening, thin, circular colonies develop after 3 to 4 days.

The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition does not takes place. Colonies are surrounded by very narrow enzymatic zones of cellulose dissolution ; attack on the cellulose takes place only in the immediate neighborhood of organisms.

Broth : No growth.

Sea water broth : No growth.

Glucose broth : No growth.

Sea water glucose broth : Turbid.

Gelatin : No growth.

Sea water gelatin : No growth.

Agar plate : No growth.

Sea water agar plate : No growth.

Glucose agar plate : No growth.

Starch agar plate : No growth.

Glucose agar plate prepared with sea water : Creamy white, semi-opaque, glistening, thin, circular colonies develop.

Glucose agar slant prepared with sea water : Creamy white, glistening, semi-opaque, thin colonies develop exclusively around the condensed water.

Starch agar plate prepared with sea water : The same as glucose agar plate prepared with sea water.

Starch agar slant prepared with sea water : The same as glucose agar slant

prepared with sea water.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Creamy white, glistening, semi-opaque, thin, circular colonies develop.

Glucose mineral agar slant prepared with sea water: Growth becomes apparent exclusively around the condensed water. Creamy white, glistening, semi-opaque, thin, beaded.

Starch mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrate rapidly reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Xylose, arabinose, glucose, fructose, galactose, mannose, cellobiose, maltose, lactose, cellulose, and starch.

Not utilized: Rhamnose, trehalose, sucrose, raffinose, inulin, agar, glycerin, erythritol, mannitol, inositol, dulcitol, salicin, Ca-lactate, Ca-gluconate, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salt concentration range for growth: Growth occurs exclusively on media prepared with natural sea water. No growth occurs on media prepared with NaCl solution isotonic with sea water.

Optimum concentration of NaCl for growth: Maximum growth occurrs on media prepared with natural sea water having a salinity of 3 to 4 per cent.

pH range for growth: 5.0–8.0.

Optimum pH: 7.0.

Temperature range for growth: 20–35°C.

Optimum temperature: 25°C.

Thermal death point: 50°C for 5 minutes.

Aerobic.

Source: Eight cultures were isolated from sea water collected from Maizuru Bay and cotton fishing nets submerged in sea water in Hiroshima Bay.

Habitat: The sea.

Vibrio macerans nov. sp.

(Pl. 7)

Etymology: Latin macerans, softening, macerating.

Morphology

Vegetative cells: Slightly curved or straight slender rigid rods, with rounded ends. Cells 0.35 to 0.4 microns broad by 3.0 to 5.0 microns long. Actively motile with a single polar flagellum. Cells show a spiral forward movement. Occurring singly and rarely in pairs. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth becomes apparent after 3 to 4 days (at 25°C) on the paper at the air-water interface or a little above it. The growth area has a tendency to spread upwards, but there is no visible development below water level. No pigment produced on the paper. The liquid becomes turbid and semi-opaque.

Cellulose is rapidly decomposed. The attacked filter paper becomes weak and macerated, but there is never complete lysis. On shaking, the attacked paper breaks up into particles and shreds of fibers.

On mineral agar plates (prepared with sea-water) containing finely divided precipitated cellulose, ashen white to milk white, opaque, thin, circular colonies with undulate margins appear in 3 to 4 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition apparently does not take place. Narrow enzymatic zones of cellulose dissolution appear around colonies after 10 days or more.

Broth: No growth.

Sea water broth: No growth.

Glucose broth: No growth.

Sea water glucose broth: Slightly turbid.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: No growth.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: Ashen white, glistening, opaque, flat, circular colonies develop. Agar is not decomposed.

Glucose agar slant prepared with sea water: Ashen white, glistening, opaque, flat, feeble colonies appear around the condensed water.

Starch agar plate prepared with sea water: The same as glucose agar plate prepared with sea water.

Starch agar slant prepared with sea water: Feeble growth, ashen white, glistening, semi-opaque, flat beaded colonies appear around and a little above the condensed water.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Ashen white to milk white, opaque, glistening, thin, circular colonies develop.

Glucose mineral agar slant prepared with sea water: Ashen white to milk white, opaque, glistening, thin, beaded colonies appear a little above the condensed water.

Starch mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrite not produced from nitrate in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Xylose, arabinose, glucose, galactose, cellobiose, maltose, lactose, trehalose, sucrose, raffinose, cellulose, starch, inulin, glycerin, inositol, salicin, and Ca-gluconate.

Not utilized: Rhamnose, fructose, mannose, agar, erythritol, mannitol, dulcitol,

Ca-lactate, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $NaNO_2$, $(NH_4)_2SO_4$, NH_4Cl, histidine, and peptone.

Not utilized: Urea and aspartic acid.

Salt concentration range for growth: 1.0–5.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water having a salinity of 3 to 4 per cent.

pH range for growth: 6.0–8.0.

Optimum pH: 7.0.

Temperature range for growth: 20–35°C.

Optimum temperature: 30°C.

Thermal death point: 60°C for 30 minutes.

Source: Two cultures were isolated from fishing nets submerged in sea water in Maizuru Bay.

Habitat: The sea.

Vibrio marinoliquefaciens nov. sp.

(Pl. 8)

Etymology: Latin marinus, pertaining to the sea, and liquefacio, to make liquid.

Morphology

Vegetative cells: Curved rigid rods, with rounded ends, usually C-shaped, with occasional S-shaped form. Cells 0.3 to 0.4 microns broad by 2.2 to 3.8 microns long. Actively motile with a single polar flagellum. Cells show a spiral forward movement. Occurring singly and rarely in pairs. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics.

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth becomes apparent after 2 to 3 days (at 25°C) as a creamy white area on the paper at the air-water interface or a little above it. The growth area has a tendency to spread upwards, but there is no visible development below water level. Pigment produced is non water-soluble. The liquid becomes turbid and semi-opaque. Cellulose is rapidly decomposed. The attacked filter paper becomes weak and macerated, but there is never complete lysis. On shaking, the attacked paper breaks up into particles and shreds of fibers.

On mineral agar plates (prepared with sea water) containing finely divided precipitated cellulose, creamy white, semi-opaque, glistening, thin, circular colonies appear

after 3 to 4 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition apparently does not take place. Colonies are not surrounded by enzymatic zones of cellulose dissolution; attack on the cellulose takes place only in the immediate neighborhood of organisms.

Broth: Slightly turbid.

Sea water broth: Turbid.

Glucose broth: Slightly turbid.

Sea water glucose broth: Turbid.

Gelatin stab: Creamy white, semi-opaque, circular, small colony develops on the surface.

Sea water gelatin stab: Stratiform liquefaction.

Agar plate: Creamy white, glistening, semi-opaque, circular, small colonies with undulate margin develop.

Sea water agar plate: Creamy white, glistening, semi-opaque, circular colonies with undulate margin develop.

Glucose agar plate: The same as agar plate.

Starch agar plate: The same as agar plate.

Glucose agar plate prepared with sea water: The same as sea water agar plate. Agar is not decomposed.

Glucose agar slant prepared with sea water: Abundant growth, creamy white, glistening, semi-opaque, flat, echinulate.

Starch agar plate prepared with sea water: The same as sea water agar plate.

Starch agar slant prepared with sea water: The same as glucose agar slant prepared with sea water.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Creamy white, glistening, semi-opaque, circular colonies develop.

Glucose mineral agar slant prepared with sea water: Creamy white, glistening, semi-opaque, echinulate. Gas is produced in the condensed water.

Starch mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No change.

Nitrite not produced from nitrate in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

Nitrate not reduced to nitrite in nitrate broth prepared with sea water.

NH_3 rapidly formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed and also in sea water broth.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed and in sea water broth.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed and in sea water broth.

Utilizable carbon sources: Xylose, arabinose, rhamnose, glucose, fructose, galactose, mannose, cellobiose, maltose, lactose, trehalose, sucrose, raffinose, cellulose, starch, glycerin, erythritol, mannitol, inositol, salicin, Ca-lactate, Ca-gluconate, and Na-alginate.

Not utilized: Inulin and agar.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salt concentration range for growth: 0.5–5.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 6.0–8.0.

Optimum pH: 7.0.

Temperature range for growth: 20–35°C.

Optimum temperature: 30°C.

Thermal death point: 50°C for 5 minutes.

Aerobic.

Source: Two cultures were isolated from sea water collected in Maizuru Bay.

Habitat: The sea.

Vibrio simplex nov. sp.

(Pl. 9)

Etymology; Latin simplex, simple.

Morphology

Vegetative cells: Curved or slightly curved short rigid rods, with rounded ends. Cells 0.5 to 0.6 microns broad by 2 to 3 microns long. Actively motile by means of a single polar flagellum. Cells show a spiral forward movement. Occurring singly and rarely in pairs. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics.

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth occurs as a pale yellow area on the paper at the air-water interface (after 3 to 4 days at 25°C). There is no visible development below the water level, but so long as the upper part of the strip remains moist the growth will spread on the paper above the liquid surface. The liquid becomes turbid and semi-opaque. After 5 to 7 days the growth area becomes rust brown. The pigment produced is non water-soluble.

After prolonged incubation the filter paper at water level becomes gradually dissolved. Although fibrolysis is much less marked than with cytophagas, the action on cellulose is lytic rather than macerative.

On mineral agar plate (prepared with sea water) containing finely divided precipitated cellulose, pale brownish yellow, semi-opaque, glistening, thin, circular colonies with undulate margin appear after 4 to 5 days.

The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition does not take place. The colonies are surrounded by clear enzymatic zones of cellulose dissolution.

Broth: No growth.

Sea water broth: No growth.

Glucose broth: No growth.

Sea water glucose broth: No growth.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: No growth.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: No growth.

Starch agar plate prepared with sea water: No growth.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Pale brownish yellow, semi-opaque, glistening, thin, circular colonies with undulate margin develop.

Glucose mineral agar slant prepared with sea water: Pale brownish yellow, semi-opaque, glistening, thin colonies appear exclusively around the condensed water.

Starch mineral agar plate prepared with sea water: No growth.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato : No growth.

Milk : No growth.

Nitrate is rapidly reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources : Xylose, glucose, cellobiose, and cellulose.

Not utilized : Arabinose, rhamnose, fructose, galactose, mannose, maltose, lactose, trehalose, sucrose, raffinose, starch, inulin, agar, glycerin, erythritol, mannitol, inositol, dulcitol, salicin, Ca-lactate, Ca-gluconate, and Na-alginate.

Utilizable nitrogen sources : $NaNO_3$, $(NH_4)_2SO_4$, and NH_4Cl.

Not utilized : $NaNO_2$, urea, aspartic acid, histidine, and peptone.

Salt concentration range for growth : 3.0–5.0%.

Optimum concentration of NaCl for growth : 3.0%.

Maximum growth obtained in media prepared with natural sea water having a salinity of 3.0 to 4.0 per cent.

pH range for growth : 6.0–9.0.

Optimum pH : 7.0–8.0.

Temperature range for growth : 15–30°C.

Optimum temperature : 25°C.

Thermal death point : 50°C for 5 minutes.

Source : Five cultures were isolated from sea water collected from Maizuru Bay.

Habitat : The sea.

Vibrio ferrugineus nov. sp.

(Pl. 10)

Etymology : Latin ferrugineus, resembling rust.

Morphology

Vegetative cells : Curved or slightly curved rigid rods, with rounded ends, 0.35 to 0.4 by 2.0 to 3.0 microns. Actively motile by means of a single polar flagellum. Occurring singly. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics

Growth on cellulose : In tubes of liquid mineral medium (prepared with sea

water) with a strip of filter paper partly immersed, growth occurs as a rust brown area on the paper at the air-water interface. There is no visible development below the water level, but so long as the upper part of the strip remains moist the growth will spread on the paper above the liquid surface. The pigment produced is non water-soluble. After prolonged incubation the filter paper at water level becomes gradually dissolved. The growth area has a tendency to become semi-transparent after prolonged incubation. The liquid becomes turbid and semi-opaque. The action on cellulose is lytic rather than macerative.

On mineral agar plate (prepared with sea water) containing finely divided precipitated cellulose, brownish yellow, semi-opaque, glistening, thin, circular colonies develop after 4 to 5 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition does not take place. The colonies are surrounded by clear enzymatic zones of cellulose dissolution.

Broth : No growth.
Sea water broth : No growth.
Glucose broth : No growth.
Sea water glucose broth : No growth.
Gelatin : No growth.
Sea water gelatin : No growth.
Agar plate : No growth.
Sea water agar plate : No growth.
Glucose agar plate : No growth.
Starch agar plate : No growth.
Glucose agar plate prepared with sea water : No growth.
Starch agar plate prepared with sea water : No growth.
Mineral agar plate prepared with sea water : No growth.
Glucose mineral agar plate prepared with sea water : No growth.
Starch mineral agar plate prepared with sea water : No growth.
Cellobiose mineral agar plate prepared with sea water : No growth.
Potato : No growth.
Milk : No growth.

Nitrite not produced from nitrate in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources : Glucose, cellobiose, and cellulose,

Not utilized: Xylose, arabinose, rhamnose, fructose, galactose, mannose, maltose, lactose, trehalose, sucrose, raffinose, starch, inulin, agar, glycerin, erythritol, mannitol, inositol, dulcitol, salicin, Ca-lactate, Ca-gluconate, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salt concentration range for growth: 1.0–5.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water having a salinity of 3.0 to 4.0 per cent.

pH range for growth: 6.0–9.0.

Optimum pH: 7.0–8.0.

Temperature range for growth: 15–30°C.

Optimum Temperature: 25°C.

Thermal death point: 50°C for 5 minutes.

Source: Eight cultures were isolated from sea water collected from Maizuru Bay and fishing nets immersed in sea water in Hiroshima Bay.

Habitat: The sea.

Vibrio brevis nov. sp.
(Pl. 11)

Etymology: Latin brevis, short.

Morphology

Vegetative cells: Curved short rigid rods, with rounded ends. Cells 0.6 to 0.8 microns broad by 1.5 to 2.0 microns long. Actively motile by means of a single polar flagellum. Cells show a spiral forward movement. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth occurs as a cream yellow area on the paper at the air-water interface (after 2 to 3 days at 25°C). There is no visible development below the water level, but so long as the upper part of the strip remains moist the growth will spread on the paper above the liquid surface. The liquid becomes turbid and semi-opaque. After prolonged incubation the growth area becomes rust brown and semi-opaque. The pigment produced is not water-soluble. The action on cellulose is lytic rather than macerative.

On mineral agar plate (prepared with sea water) containing finely divided precipitated cellulose, creamy, semi-opaque, glistening, thin, circular colonies appear

after 4 to 5 days.

The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition does not take place. The colonies are surrounded by narrow clear zones of cellulose dissolution.

Broth: No growth.

Sea water broth: Turbid.

Glucose broth: No growth.

Sea water glucose broth: Strongly turbid.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: Cream-colored, glistening, circular, small colonies develop.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: Cream-colored, glistening, circular, small colonies develop.

Glucose agar slant prepared with sea water: Cream-colored glistening, beaded.

Starch agar plate prepared with sea water: The same as glucose agar plate prepared with sea water.

Starch agar slant prepared with sea water: Creamy white, glistening, beaded.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Pale yellow, glistening, circular, thin, small colonies develop.

Glucose mineral agar slant prepared with sea water: Pale yellow, glistening, thin, beaded.

Starch mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrate not reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed or in sea water nitrate broth.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with

filter paper partly immersed.

H_2S formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Arabinose, glucose, fructose, galactose, mannose, cellobiose, trehalose, cellulose, mannitol, inositol, and Ca-gluconate.

Not utilized: Xylose, rhamnose, maltose, lactose, sucrose, raffinose, starch, inulin, agar, glycerin, erythritol, dulcitol, salicin, Ca-lactate, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilizd: $NaNO_2$.

Salinity range for growth: 3.0–5.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water having a salinity of 3.0 to 4.0 per cent.

pH range for growth: 6.0–9.0.

Optimum pH: 8.0.

Temperature range for growth: 15–30°C.

Optimum temperature: 25°C.

Thermal death point: 50°C for 10 minutes.

Source: Nine cultures were isolated from sea water and fishing nets immersed in sea water in Maizuru Bay.

Habitat: The sea.

Vibrio fulvus nov. sp.

(Pl. 12)

Etymology: Latin fulvus, yellowish brown.

Morphology

Vegetative cells: Markedly curved rigid rods, usually C-shaped, with occasional S-shaped forms. Cells 0.4 microns broad by 1.8 to 3.5 microns long. Actively motile by means of a single polar flagellum. Cells show a spiral forward movement. Occurring singly and in pairs. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth occurs as a yellowish brown area on the paper at the air-water interface (after 3 to 4 days at 25°C). There is no visible development below the water level, but so long as the upper part of the strip remains moist the growth will spread on the paper above the liquid surface.

The liquid becomes turbid and semi-opaque. The pigment produced is non water-soluble. After prolonged incubation the filter paper at water level becomes rust brown and semi-opaque.

The action on cellulose is lytic rather than macerative.

On mineral agar plate (prepared with sea water) containing finely divided precipitated cellulose, pale yellow-brown, semi-opaque, glistening, thin, circular colonies with entire margin appear after 4 to 5 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition does not take place. The colonies are surrounded by enzymatic clear zones of cellulose-dissolution.

Broth: Slightly turbid.

Sea water broth: Strongly turbid.

Glucose broth: Slightly turbid.

Sea water glucose broth: Heavy turbidity, no pellicle.

Gelatin stab: Pale yellow, semi-opaque, glistening, circular, small colony develops on the surface. No liquefaction.

Sea water gelatin stab: Pale yellow, semi-opaque, glistening, circular colony develops on the surface. No liquefaction.

Agar plate: Pale yellow, semi-opaque, glistening, circular, small colonies with entire margin develop.

Sea water agar plate: Pale yellow, semi-opaque, glistening, circular colonies with entire margin develop.

Sea water agar slant: Abundant growth. Pale yellow, semi-opaque, glistening, echinulate.

Glucose agar plate: The same as agar plate.

Starch agar plate: The same as agar plate.

Glucose agar plate prepared with sea water: The same as sea water agar plate.

Starch agar plate prepared with sea water: The same as sea water agar plate.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Pale yellow, semi-opaque, glistening, circular colonies develop.

Glucose mineral agar slant prepared with sea water: Pale yellow, semi-opaque, glistening, echinulate.

Starch mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrate reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed and in sea water nitrate broth.

NH_3 formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed or in sea water broth.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Xylose, arabinose, glucose, fructose, galactose, mannose, cellobiose, lactose, sucrose, raffinose, cellulose, glycerin, mannitol, salicin, Ca-lactate, and Ca-gluconate.

Not utilized: Rhamnose, maltose, trehalose, starch, inulin, agar, erythritol, inositol, dulcitol, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salinity range for growth: 0.5–5.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water having a salinity of 3.0 to 4.0 per cent.

pH range for growth: 6.0–9.0.

Optimum pH: 7.0–8.0.

Temperature range for growth: 15–30°C.

Optimum temperature: 20–25°C.

Thermal death point: 50°C for 5 minutes.

Source: Twenty nine cultures were isolated from sea water collected in Maizuru Bay and Hiroshima Bay.

Habitat: The sea.

Vibrio aurantiacus nov. sp.

(Pl. 13)

Etymology: M. L., aurantiacus, orange-colored.

Morphology

Vegetative cells: Curved rigid rods, usually C-shaped, with occasional S-shaped forms. Cells 0.35 microns broad by 3.0 to 4.5 microns long.

Actively motile by means of a single polar flagellum. Cells show a spiral

forward movement. Occurring singly and in pairs. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth occurs as a deep yellow to orange yellow area on the paper at the air-water interface (after 3 to 4 days at 25°C). There is no visible development below the water level, but so long as the upper part of the strip remains moist the growth will spread on the paper above the liquid surface. The liquid becomes turbid and semi-opaque. Yellow mucilaginous pellicle develops. The pigment produced is not water-soluble. After prolonged incubation the filter paper at water level becomes gradually semi-opaque.

The action on cellulose is lytic rather than macerative.

On mineral agar plate (prepared with sea water) containing finely divided precipitated cellulose, yellow, semi-opaque, glistening, thin, circular colonies appear after 4 to 5 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition does not take place. The colonies are surrounded by distinct clear zones of cellulose dissolution.

Broth: No growth.

Sea water broth: No growth.

Glucose broth: No growth.

Sea water glucose broth: Turbid.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: No growth.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: Cream-yellow, semi-opaque, glistening, circular colonies develop.

Glucose agar slant prepared with sea water: Cream-yellow, semi-opaque, glistening, echinulate.

Starch agar plate prepared with sea water: No growth.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Pale yellow, translucent, glistening, thin, circular colonies develop.

Glucose mineral agar slant prepared with sea water: Pale yellow, translucent, glistening, thin, echinulate.

Starch mineral agar plate prepared with sea water: No growth.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrate not reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Xylose, glucose, mannose, cellobiose, sucrose, and cellulose.

Not utilized: Arabinose, rhamnose, fructose, galactose, maltose, lactose, trehalose, raffinose, starch, inulin, agar, glycerin, erythritol, mannitol, inositol, dulcitol, salicin, Ca-lactate, Ca-gluconate, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salinity range for growth: 1.0–5.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water having a salinity of 3.0 to 4.0 per cent.

pH range for growth: 6.0–9.0.

Optimum pH: 7.0–8.0.

Temperature range for growth: 20–30°C.

Optimum temperature: 25°C.

Thermal death point: 50°C for 10 minutes.

Source: Twelve cultures were isolated from sea water collected from Maizuru Bay and Hiroshima Bay.

Habitat: The sea.

Cellvibrio sp. isolated by HIDAKA[8] from digestive organs of *Teredo navalis* sp. resembles *Vibrio aurantiacus*, but the former differs from the latter in respects of size of cells, and the utilization of several carbon and nitrogen sources.

Vibrio marinotypicus nov. sp.

(Pl. 14)

Etymology: Latin marinus, of the sea and typicus, typical.

Morphology

Vegetative cells: Curved rigid rods, with rounded ends. Cells 0.3 to 0.35 microns broad by 1.2 to 2.4 microns long. Actively motile by means of a single polar flagellum. Cells show a spiral forward movement. Occurring singly and in pairs. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics.

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth occurs as a rust brown area on the paper at the air-water interface (after 3 to 4 days at 25°C). There is no visible development below the water level, but so long as the upper part of the strip remains moist the growth will spread on the paper above the liquid surface. The liquid becomes turbid. The pigment produced is non water-soluble. After prolonged incubation the filter paper at water level becomes semi-opaque. The action on cellulose is lytic rather than macerative.

On mineral agar plate (prepared with sea water) containing finely divided precipitated cellulose, cream colored, semi-translucent, glistening, thin, circular colonies appear after 4 to 5 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition does not take place. The colonies are surrounded by narrow enzymatic clear zones of cellulose dissolution.

Broth: No growth.

Sea water broth: Heavy turbidity. No pellicle.

Glucose broth: No growth.

Sea water glucose broth: Strongly turdid. No pellicle.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: Creamy white, semi-translucent, glistening, thin, circular colonies develop.

Sea water agar slant: Abundant growth, milky white, semi-translucent, glistening, thin, echinulate.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: Creamy white, semi-translucent, glistening, thin, circular colonies develop.

Glucose agar slant prepared with sea water: The same as sea water agar slant.

Starch agar plate prepared with sea water: The same as glucose agar plate prepared with sea water.

Starch agar slant prepared with sea water: The same as glucose agar slant prepared with sea water.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Cream colored, semi-translucent, thin, circular colonies develop.

Glucose mineral agar slant prepared with sea water: Cream colored, semi-translucent, thin, echinulate, with undulate margin.

Starch mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrate is reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed, but nitrite is not formed from nitrate in sea water nitrate broth.

NH_3 produced from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Xylose, glucose, fructose, galactose, mannose, cellobiose, maltose, lactose, trehalose, sucrose, raffinose, starch, cellulose, glycerin, salicin, Ca-lactate, and Na-alginate.

Not utilized: Arabinose, rhamnose, inulin, agar, erythritol, mannitol, inositol, and Ca-gluconate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salinity range for growth: 1.0–5.0%.

Optimum salinity for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 6.0–9.0.
Optimum pH: 7.0–8.0.
Temperature range for growth: 20–30°C.
Optimum temperature: 25°C.
Thermal death point: 50°C for 20 minutes.
Source: Sixteen cultures were isolated from sea water collected from Maizuru Bay and Hiroshima Bay.
Habitat: The sea.

Vibrio euryhalis nov. sp.
(Pl. 15)

Etymology: Greek eury, broad, and halinus, salty; adapted to a broad change of salinity.

Morphology

Vegetative cells: Markedly curved rigid rods, with rounded ends. Usually C-shaped, with occasional S-shaped forms. Cells 0.3 to 0.35 microns broad by 1.8–2.4 microns long. Actively motile by means of a single polar flagellum. Cells show a spiral forward movement. Occurring singly and in pairs. Gram negative. Stain uniformly with GIEMSA's stain.
No spores.

Cultural and physiological characteristics.

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth occurs as a rust brown area on the paper at the air-water interface (after 3 to 4 days at 25°C). There is no visible development below the water level, but so long as the upper part of the strip remains moist the growth will spread on the paper above the liquid surface. The liquid becomes turbid and semi-opaque. The pigment produced is non water-soluble. After prolonged incubation the filter paper at water level becomes semi-opaque. The action on cellulose is lytic rather than macerative.

On mineral agar plate (prepared with sea water) containing finely divided precipitated cellulose, brownish yellow, semi-translucent, glistening, thin, circular colonies with undulate margin appear after 4 to 5 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition does not take place. The colonies are surrounded by enzymatic clear zones of cellulose-dissolution.

Broth: Slightly turbid.
Sea water broth: Heavy turbidity.
Glucose broth: Slightly turbid.

Sea water glucose broth: Heavy turbidity.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: Creamy white, opaque, glistening, circular, small colonies with undulate margin develop.

Sea water agar plate: Creamy white, opaque, glistening, circular colonies with undulate margin develop.

Sea water agar slant: Abundant growth, creamy white, opaque to semi-opaque, glistening, filiform.

Glucose agar plate: The same as agar plate.

Starch agar plate: The same as agar plate.

Glucose agar plate prepared with sea water: The same as sea water agar plate.

Starch agar plate prepared with sea water: The same as sea water agar plate.

Glucose agar slant prepared with sea water: The same as sea water agar slant.

Starch agar slant prepared with sea water: The same as sea water agar slant.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Milky white, semi-translucent, glistening, circular.

Glucose mineral agar slant prepared with sea water: Milky white, semi-translucent, glistening, echinulate.

Starch mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrate reduced to nitrite in sea water nitrate broth.

NH_3 formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed or sea water broth.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Xylose, arabinose, rhamnose, glucose, fructose, galactose, mannose, cellobiose, maltose, lactose, trehalose, sucrose, cellulose, starch, glycerin, erythritol, mannitol, inositol, salicin, Ca-lactate, and Ca-gluconate.

Not utilized: Raffinose, inulin, agar, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salinity range for growth: 0.0 to 5.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water having a salinity of 3.0 to 4.0%.

pH range for growth: 5.0–9.0.

Optimum pH: 7.0–8.0.

Temperature range for growth: 20–35°C.

Optimum temperature: 25°C.

Thermal death point: 50°C for 40 minutes.

Source: Four cultures were collected from sea water collected from Maizuru Bay.

Habitat: The sea.

Vibrio marinoflavescens KADOTA.[32)50)]

(Pl. 16)

Vegetative cells: Markedly curved rigid rods, with rounded ends, usually C-shaped, with occasional S-shaped forms. Cells 0.35 to 0.4 microns broad by 1.5 to 2.0 microns long. Actively motile by means of a single polar flagellum. Cells show a spiral forward movement. Occurring singly and in pairs. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth occurs as a brownish yellow area on the paper at the air-water interface (after 3 to 4 days at 25°C). There is no visible development below the water level, but so long as the upper part of the strip remains moist the growth will spread on the paper above the liquid surface. The liquid becomes turbid and semi-opaque. The pigment produced is non water-soluble. The pigment produced on the paper above the liquid surface becomes brown after 5 to 7 days. After prolonged incubation the filter paper at water level becomes semi-opaque. The action on cellulose is lytic rather than macerative.

On mineral agar plate (prepared with sea water) containing finely divided precipitated cellulose, pale yellow, semi-translucent, glistening, thin, circular colonies with entire margin appear after 4 to 5 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar decomposition does not take place. The colonies are surrounded by indistinct enzymatic clear zones of cellulose dissolution.

Broth: Turbid. No pellicle.

Sea water broth: Heavy turbidity.

Glucose broth: Turbid. No pellicle.

Sea water glucose broth: Heavy turbidity.

Gelatin: Pale yellow, semi-translucent, glistening, thin, circular colony develops on the surface. No liquefaction.

Sea water gelatin: Pale yellow, semi-translucent, glistening, thin, circular colony develops on the surface. No liquefaction.

Agar plate: Pale yellow, semi-translucent, glistening, thin, circular colonies develop.

Sea water agar plate: Pale yellow, semi-opaque, glistening, thin, circular colonies develop.

Sea water agar slant: Pale yellow, semi-opaque, glistening, echinulate.

Glucose agar plate: The same as agar plate.

Starch agar plate: The same as agar plate.

Glucose agar plate prepared with sea water: Pale yellow, semi-opaque, glistening, thin, circular colonies develop.

Glucose agar slant prepared with sea water: The same as sea water agar slant.

Starch agar plate prepared with sea water: The same as glucose agar plate prepared with sea water.

Starch agar slant prepared with sea water: The same as sea water agar slant.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: Pale yellow, semi-translucent, glistening, thin, circular, small colonies develop.

Glucose mineral agar slant prepared with sea water: Pale yellow, semi-translucent, glistening, filiform.

Starch mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrate reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 produced from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Rhamnose, glucose, fructose, galactose, mannose, cellobiose, maltose, lactose, trehalose, sucrose, raffinose, cellulose, starch, inulin, glycerin, mannitol, inositol, salicin, Ca-lactate, and Ca-gluconate.

Not utilized: Xylose, arabinose, agar, erythritol, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salinity range for growth: 0.5–5.0 per cent.

Optimum salinity for growth: 3.0 per cent.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 6.0–9.0.

Optimum pH: 7.0–8.0.

Temperature range for growth: 15–30°C.

Optimum temperature: 25°C.

Thermal death point: 50°C for 20 minutes.

Source: Thirty cultures were isolated from sea water collected from Maizuru Bay and Hiroshima Bay.

Habitat: The sea.

Vibrio xanthus nov. sp.

(Pl. 17)

Etymology: Greek xanthos, deep yellow.

Morphology

Vegetative cells: Short curved rods, with rounded ends. Cells 0.55 to 0.65 microns broad by 1.2 to 2.5 microns long. Usually C-shaped, with occasional S-shaped forms. Motile by means of a single polar flagellum. Occurring singly and in pairs. Gram negative. Stain uniformly with GIEMSA's stain.

No spores.

Cultural and physiological characteristics.

Growth on cellulose : In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth occurs as a deep yellow area on the paper at the air-water interface (after 3 to 4 days at 25°C). There is no visible development below the water level, but so long as the upper part of the strip remains moist the growth will spread on the paper above the liquid surface. The pigment produced is water-soluble. The paper is deeply stained with the pigment. The liquid becomes yellow and semi-opaque. After prolonged incubation the filter paper at water level becomes semi-opaque. The action on cellulose is lytic rather than macerative.

On mineral agar plate (prepared with sea water) containing finely divided precipitated cellulose, yellow, translucent, glistening, thin, circular colonies with entire margins develop after 4 to 5 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar-decomposition does not take place. The colonies are surrounded by indinstinct enzymatic zones of cellulose dissolution.

Broth : No growth.

Sea water broth : Heavy turbidity.

Glucose broth : No growth.

Sea water glucose broth : Strongly turbid.

Gelatin : No growth.

Sea water gelatin : No growth.

Agar plate : No growth.

Sea water agar plate : Yellow, translucent, glistening, thin, circular colonies with undulate margins develop.

Sea water agar slant : Yellow, translucent, glistening, thin, filiform to echinulate.

Glucose agar plate : No growth.

Starch agar plate : No growth.

Glucose agar plate prepared with sea water : Yellow, semi-translucent, glistening, thin, circular colonies with undulate margins develop.

Glucose agar slant prepared with sea water : Yellow, semi-translucent, glistening, thin, filiform to echinulate.

Starch agar plate prepared with sea water : The same as glucose agar plate prepared with sea water.

Starch agar slant prepared with sea water : The same as glucose agar slant prepared with sea water.

Mineral agar plate prepared with sea water : No growth.

Glucose mineral agar plate prepared with sea water : Yellow, translucent, glistening, circular colonies develop.

Glucose mineral agar slant prepared with sea water: Yellow, translucent, glistening, echinulate.

Starch mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Starch mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Cellobiose mineral agar plate prepared with sea water: The same as glucose mineral agar plate prepared with sea water.

Cellobiose mineral agar slant prepared with sea water: The same as glucose mineral agar slant prepared with sea water.

Potato: No growth.

Milk: No growth.

Nitrate reduced to nitrite in liquid mineral medium (prepared with sea water) with filter paper partly immersed, and in sea water nitrate broth.

NH_3 abundantly produced from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed, and in sea water broth.

Utilizable carbon sources: Xylose, glucose, cellobiose, trehalose, sucrose, cellulose, starch, and glycerin.

Not utilized: Arabinose, rhamnose, fructose, galactose, mannose, maltose, lactose, raffinose, inulin, agar, erythritol, mannitol, inositol, dulcitol, salicin, Ca-lactate, Ca-gluconate, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salinity range for growth: 3.0–5.0%.

Optimum salinity for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 6.0–9.0.

Optimum pH: 8.0.

Temperature range for growth: 15–30°C.

Optimum temperature: 25°C.

Thermal death point: 50°C for 5 minutes.

Source: Six cultures were isolated from sea water collected from Maizuru Bay.

Habitat: The sea.

Cytophaga haloflava KADOTA.[33]

(Pl. 18)

Morphology

Vegetative cells: Long, slender, flexible, singly occurring rods, with rounded ends, 0.3 to 0.45 microns broad by 2.5 to 10.0 microns long. Length very variable. Weakly reflactile. Non-flagellate. Creeping motility on solid surfaces. Gram negative. Stain uniformly with GIEMSA's stain.

No fruiting bodies or spores (microcysts) formed.

Cultural and physiological characteristics.

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth becomes apparent after 2 to 4 days (at 25°C) as a bright yellow mucilaginous area on the paper at the air-water interface. The growth area has a tendency to spread upwards, but there is no visible development below water level. The liquid becomes turbid and semi-opaque. The pigment produced is not water-soluble. The filter paper at water level is gradually completely dissolved and the patches become translucent. The action on cellulose is lytic rather than macerative.

On mineral agar plates (prepared with sea water) containing finely divided precipitated cellulose, bright yellow, thin, glistening, translucent, mucilaginous, circular colonies with entire margin develop after 3 to 5 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar-decomposition does not take place. Colonies are not surrounded by enzymatic zones of cellulose dissolution; attack on the cellulose takes place only in the immediate neighborhood of the organisms.

Broth: No growth.

Sea water broth: No growth.

Glucose broth: No growth.

Sea water glucose broth: No growth.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: No growth.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: No growth.

Starch agar plate prepared with sea water: No growth.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: No growth.

Starch mineral agar plate prepared with sea water: No growth.

Cellobiose mineral agar plate prepared with sea water: Yellow, thin, glistening, translucent, mucilaginous, circular, faint colonies with entire margin develop after 10 days or more.

Cellobiose mineral agar slant prepared with sea water: Yellow, thin, glistening, translucent, mucilaginous faint colonies develop around the condensed water after 10 days or more.

Potato: No growth.

Milk: No growth.

Nitrite produced from nitrate in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Glucose, cellobiose, trehalose, and cellulose.

Not utilized: Xylose, arabinose, rhamnose, fructose, galactose, mannose, maltose, lactose, sucrose, raffinose, starch, inulin, agar, glycerin, erythritol, mannitol, inositol, dulcitol, salicin, Ca-lactate, Ca-gluconate, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, KNO_3, $NaNO_2$, NH_4Cl, $(NH_4)_2SO_4$, urea, aspartic acid, histidine, and peptone.

Salinity range for growth: 1.0–5.0%.

Optimum salinity for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 7.0–8.0.

Optimum pH: 7.2

Temperature range for growth: 15–30°C.

Optimum temperature: 20–25°C.

Thermal death point: 50°C for 5 minutes.

Source: Eight cultures were isolated from fishing nets immersed in Maizuru Bay and fishing nets which had been stored for three months after fishing in Hiroshima Bay.

Habitat: The sea.

Cytophaga haloflava var. *nonreductans* KADOTA

(Pl. 19)

Morphology

Vegetative cells: Long, slender, flexible, singly occurring rods, with rounded

ends, 0.3 to 0.4 microns broad by 3 to 6 microns long. Length very variable. Weakly reflactile. Non flagellate. Creeping motility on solid surfaces. Gram negative. Stain uniformly with GIEMSA's stain.

No fruiting bodies or spores (microcysts) formed.

Cultural and physiological characteristics.

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth becomes apparent after 2 to 4 days (at 25°C) as a bright yellow mucilaginous area on the paper at the air-water interface. The growth area has a tendency to spread upwards, but there is no visible development below water level. The liquid becomes turbid and semi-opaque. The pigment produced is not water-soluble. The filter paper at water level is gradually completely dissolved and the patches become translucent. The action on cellulose is lytic rather than macerative.

On mineral agar plates (prepared with sea water) containing finely divided precipitated cellulose, bright yellow, thin, glistening, translucent, mucilaginous, circular colonies with entire margin develop after 3 to 5 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar-decomposition does not take place. Colonies are not surrounded by enzymatic zones of cellulose dissolution; attack on cellulose takes place only in the immediate neighborhood of the organisms.

Broth: No growth.

Sea water broth: No growth.

Glucose broth: No growth.

Sea water glucose broth: No growth.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: No growth.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: No growth.

Starch agar plate prepared with sea water: No growth.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: No growth.

Starch mineral agar plate prepared with sea water: No growth.

Cellobiose mineral agar plate prepared with sea water: Yellow, thin, glistening, translucent, mucilaginous, circular, faint colonies with entire margin develop after 10 days or more.

Cellobiose mineral agar slant prepared with sea water: Yellow, thin, glistening, translucent, mucilaginous faint colonies develop around the condensed water after 10 days or more.

Potato: No growth.

Milk: No growth.

Nitrite not formed from nitrate in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Glucose, cellobiose, trehalose, and cellulose.

Not utilized: Xylose, arabinose, rhamnose, fructose, galactose, mannose, maltose, lactose, sucrose, raffinose, starch, inulin, agar, glycerin, erythritol, mannitol, inositol, dulcitol, salicin, Ca-lactate, Ca-gluconate, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, histidine, and peptone.

Not utilized: $NaNO_2$.

Salinity range for growth: 3.0–5.0%.

Optimum salinity for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 6.0–9.0.

Optimum pH: 7.0.

Temperature range for growth: 15–30°C.

Optimum temperature: 25°C.

Thermal death point: 50°C for 5 minutes.

Source: Twenty two cultures were isolated from sea water and fishing nets submerged in sea water in Maizuru Bay and Hiroshima Bay.

Habitat: The sea.

Cytophaga rosea KADOTA

(Pl. 20)

Morphology

Vegetative cells: Long, slender flexible, singly occurring rods, with rounded ends, 0.35 to 0.5 microns broad by 2.0 to 8.0 microns long. Length very variable. Weakly reflactile. Non flagellate. Creeping motility on solid surfaces. Gram negative. Stain uniformly with GIEMSA's stain.

No fruiting bodies or spores (microcysts) formed.

Cultural and physiological characteristics.

Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with a strip of filter paper partly immersed, growth becomes apparent after 3 to 4 days (at 25°C) as a pink mucilaginous area on the paper at the air-water interface. The growth area has a tendency to spread upwards, but there is no visible development below water level. The liquid becomes turbid and semi-opaque. The filter paper at water level is gradually completely dissolved and the patches become translucent. The action on cellulose is lytic rather than macerative.

On mineral agar plates (prepared with sea water) containing finely divided precipitated cellulose, pink, thin, glistening, translucent, mucilaginous colonies appear after 3 to 5 days. The agar beneath the colonies becomes slightly etched and sunken, although actual agar-decomposition does not take place. Colonies are not surrounded by enzymatic zones of cellulose dissolution; Attack on cellulose takes place only in the immediate neighbourhood of the organisms.

Broth: No growth.

Sea water broth: No growth.

Glucose broth: No growth.

Sea water glucose broth: No growth.

Gelatin: No growth.

Sea water gelatin: No growth.

Agar plate: No growth.

Sea water agar plate: No growth.

Glucose agar plate: No growth.

Starch agar plate: No growth.

Glucose agar plate prepared with sea water: No growth.

Starch agar plate prepared with sea water: No growth.

Mineral agar plate prepared with sea water: No growth.

Glucose mineral agar plate prepared with sea water: No growth.

Starch mineral agar plate prepared with sea water: No growth.

Cellobiose mineral agar plate prepared with sea water: Pink, thin, glistening, translucent, mucilaginous, circular, faint colonies with entire margin develop after 10 days or more.

Cellobiose mineral agar slant prepared with sea water: Faint pink colonies appear around the condensed water after 10 days or more.

Potato: No growth.

Milk: No growth.

Nitrite produced from nitrate in liquid mineral medium (prepared with sea water) with filter paper partly immersed.

NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Indol not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

H_2S not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.

Utilizable carbon sources: Glucose, cellobiose, and cellulose.

Not utilized: Xylose, arabinose, rhamnose, fructose, galactose, mannose, maltose, lactose, trehalose, sucrose, raffinose, starch, inulin, agar, glycerin, erythritol, mannitor, inositol, dulcitol, salicin, Ca-lactate, Ca-gluconate, and Na-alginate.

Utilizable nitrogen sources: $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, and peptone.

Not utilized: $NaNO_2$ and histidine.

Salinity range for growth: 3.0%; No growth in media with salt concentration of 1.0% and 5.0%.

Optimum concentration of NaCl for growth: 3.0%.

Maximum growth obtained in media prepared with natural sea water.

pH range for growth: 7.0 to 9.0.

Optimum pH: 7.2.

Temperature range for growth: 20–30°C.

Optimum temperature: 25°C.

Thermal death point: 50°C for 5 minutes.

Source: Ten cultures were isolated from sea water and cotton cords immersed in the sea.

Habitat: The sea.

2. Key to the species of marine aerobic cellulose-decomposing bacteria.

Morphological, cultural, and physiological studies of marine aerobic cellulose-decomposing bacteria indicated that their characters may be grouped as of primary or of secondary importance, taxonomically, as follows:

Characters of primary importance: Cell size, shape (curvature), flexibility, flagellation, motility, and stainability; character of colonies on a strip of filter paper partly immersed in liquid mineral medium prepared with sea water as to pigmentation, margin, and consistency; solubility of pigment produced; character of colonies on cellulose agar plate as to pigmentation, width and edge of enzymatic clear zone of cellulose dissolution; action on cellulose; action on gelatin; action on nitrate; production of ammonia, indol, and hydrogen sulfide from peptone; utilization of organic and inorganic nitrogen; utilization of xylose, arabinose, rhamnose, glucose, fructose, galactose, mannose, cellobiose, maltose, lactose, trehalose, sucrose, raffinose,

cellulose, starch, inulin, agar, glycerin, erythritol, mannitol, inositol, dulcitol, salicin, Ca-lactate, Ca-gluconate, and Na-alginate; temperature range for growth; salinity range for growth; pH range for growth.

Characters of secondary importance: Cell arrangement; broth characters; glucose broth characters; agar plate characters; agar slant characters; glucose agar plate characters; glucose agar slant characters; mineral agar plate characters; mineral agar slant characters; starch mineral agar plate characters; starch mineral agar slant characters; glucose mineral agar plate characters; glucose mineral agar slant characters; cellobiose mineral agar plate characters; cellobiose mineral agar slant characters; action on potato; action on litmus milk; thermal death point.

On the basis of morphology all species studied herein were divided into three main groups: (1) Gram-negative, straight, polarly flagellated rigid rods; (2) Gram-negative, curved, polarly flagellated rigid rods; and (3) Gram-negative, long, non-flagellated flexible rods showing creeping motility on solid surfaces.

According to BERGEY's Manual of Determinative Bacteriology 6th edition[51] it is clear that the first group belongs to genus *Pseudomonas*, the second group belongs to genus *Vibrio*, and the third group belongs to genus *Cytophaga*.

Both the genus *Pseudomonas* and the genus *Vibrio* are included in family *Pseudomonadaceae* and have many characters in common with each other. The first cardinal character of the genus *Vibrio* is spiral curvature of cells. Although cell curvature was often found in youg cultures only, cells of all species assigned to *Vibrio* in the present study were more or less spirally curved. STANIER[29] and HUMM[31] also studied several marine species included in the genus *Vibrio*, in which the cells were slightly curved in young cultures only. The second cardinal character of the genus *Vibrio* listed in BERGEY's Manual is possession of a single polar flagellum. Since many species of *Pseudomonas* are also said to have a single polar flagellum, this character is of little value in separating *Pseudomonas* and *Vibrio*.

Since the species included to the genus *Vibrio* in the present study decomposed cellulose without forming oxycellulose[50] they were clearly distinguished from the species belonging to the genus *Cellvibrio* or to the genus *Cellfalcicula*. The cardinal character of *Cellvibrio* and *Cellfalcicula* described in BERGEY's Manual is the ability to oxidize cellulose, forming oxycellulose.

By use of the primary characters listed above and by occasional supplementary use of secondery characters, the following keys to all species of marine aerobic cellulose-decomposing bacteria (belonging to all three genera) studied herein have been constructed.

A. Key to the species included in the genus *Pseudomonas*.

I. Soil and fresh water forms with a few that are pathogenic on cold or warm blooded animals.
II. Sea water to brine species. Some species phosphorescent.

A. Utilizes cellulose.
  1. Does not utilize agar.
     1. *Pseudomonas marinocellulosae* nov. sp.
  2. Utilize agar.
     a. Gelatin not liquefied.
        2. *Pseudomonas iridescens* STANIER.
     aa. Gelatin liquefied.
        b. Nonmotile.
           3. *Pseudomonas droebachense* (LUNDESTAD) STANIER.
        bb. Motile
           4. *Pseudomonas elongata* HUMM.

B. Do not utilize cellulose.

B. Key to the species included in the genus *Vibrio*.

I. Soil and fresh water forms with a few that are pathogenic on animals. (not obligately halophilic).

II. Marine forms (obligately halophilic).
  A. Utilize cellulose, but do not form oxycellulose.
    1. Slightly curved, slender rods of variable length. The action on cellulose is macerative rather than lytic. Good growth at 35°C.
      a. Agar digested.
        b. Gelatin liquefied.
          c. Colonies yellow; yellowish-brown water-soluble pigment produced.
            1. *Vibrio fuscus* STANIER.
          cc. Colonies white; water-soluble pigment absent.
            2. *Vibrio granii* (LUNDESTAD) STANIER.
        bb. Gelatin not liquefied.
          c. H_2S produced.
            d. Nitrite produced.
              3. *Vibrio agar-liquefaciens* (GRAY and CHALMERS) BERGEY et al.
            dd. Nitrite not produced.
              4. *Vibrio stanierii* HUMM.
          cc. H_2S not produced.
            d. Nitrite produced.
              e. Dark purple pigment produced on filter paper.
                5. *Vibrio purpureus* KADOTA.
              ee. No pigment produced on filter paper.
                5a. *Vibrio purpureus* var. *albus* KADOTA.
            dd. Nitrite not produced.
              e. Nitrite utilized as nitrogen source.
                6. *Vibrio notus* HUMM.
              ee. Nitrite not utilized as nitrogen source.
                7. *Vibrio aquamarinus* nov. sp.
      aa. Agar not digested.
        b. Gelatin not liquefied.
          c. Ammonium salt not utilized as nitrogen source.
            8. *Vibrio gilvus* nov. sp.
          cc. Ammonium salt utilized as nitrogen source.
            d. Pale yellow pigment produced on filter paper.

9. *Vibrio albogilvus* nov. sp.

dd. No pigment produced on filter paper.

10. *Vibrio macerans* nov. sp.

bb. Gelatin liquefied.

11. *Vibrio marinoliquefaciens* nov. sp.

2. Markedly curved, short rods. The action on cellulose is lytic rather than macerative. (A filter paper strip being partly immersed in liquid medium is slowly attacked and becomes translucent at the air-water interface.) No growth at 35°C.

a. Water soluble pigment not produced.

b. Starch not utilized.

c. Sucrose not utilized.

d. Organic nitrogen not utilized.

12. *Vibrio simplex* nov. sp.

dd. Organic nitrogen utilized.

e. H_2S not produced.

13. *Vibrio frrugineus* nov. sp.

ee. H_2S produced.

14. *Vibrio brevis* nov. sp.

cc. Sucrose utilized.

d. Yellowish brown to rust brown pigment produced. Mannitol and glycerin utilized. NH_3 produced.

15. *Vibrio fulvus* nov. sp

dd. Intence orange pigment produced. Mannitol and glycerin not utilized. NH_3 not produced.

16. *Vibrio aurantiacus* nov. sp.

bb. Starch utilized.

c. Mannitol not utilized.

17. *Vibrio marinotypicus* nov. sp.

cc. Mannitol utilized.

d. Raffinose not utilized.

18. *Vibrio euryhalis* nov. sp.

dd. Raffinose utilized.

19. *Vibrio marinoflavescens* KADOTA.

aa. Water soluble yellow pigment produced.

20. *Vibrio xanthus* nov. sp.

B. Do not utilize cellulose.

C. Key to the species included in the genus *Cytophaga*.

I. From soil.

II. From sea water.

A. Do not utilize agar.

1. Produce yellow pigment on cellulose.

a. Nitrate reduced.

1. *Cytophaga haloflava* KADOTA.

aa. Nitrate not reduced.

1a. *Cytophaga haloflava* var. *nonreductans* KADOTA.

2. Produces pink pigment on cellulose.

2. *Cytophaga rosea* KADOTA.

B. Utilize agar.

1. Produces dark pigment on cellulose.

3. *Cytophaga krzemieniewskae* STANIER.

2. No pigment on cellulose.

4. *Cytophaga diffluens* STANIER.

3. Produces light orange pigment on cellulose.

5. *Cytophaga sensitiva* HUMM.

III. Distribution of aerobic cellulose-decomposing bacteria in the sea.

Although, compared with land, the marine environment has a relatively high uniformity in chemical composition and other properties, bacteria are not evenly distributed in the sea. A multiplicity of interrelated factors, such as temperature, salinity, pH value, dissolved organic matter, suspended solids, etc. are thought to be responsible for the number and variety of bacteria in the sea. The number, the variety, and the activity of cellulose-decomposing bacteria in the sea are also thought to be influenced by various environmental factors.

It is, therefore, probable that the distribution of cellulose-decomposing bacteria vary seasonally, vertically and geographically in the sea. In the following experiments, variations in the seasonal, vertical, and geographical distribution of cellulose-decomposing bacteria in the sea were detected quantitatively and qualitatively.

1. Seasonal distribution of aerobic cellulose-decomposing bacteria.

As shown previously, the deterioration of cotton nettings exposed to the sea during the summer months is much more rapid than during the winter. This seasonal cycle is probably attributable to the seasonal variations in the population and the activity of cellulose-decomposing bacteria in the sea.

The present experiments were undertaken to provide experimental evidence concerning the seasonal variation in the population of cellulose-decomposing bacteria in the sea.

(1) Seasonal distribution of total cellulose-decomposing bacteria.

Samples of sea water were collected from different depths in Maizuru Bay one to three times a month for bacteriological analysis from May 1950 to December 1950 and from February 1954 to May 1954, using ZoBELL's bacteriological water sampler.[52]

For estimating the total number of aerobic cellulose-decomposing bacteria in sea water, the extinction dilution method was employed. In addition to the bacteriological examination, the chlorinity and the temperature of water samples were determined coincidentaly.

The results obtained were given in Table 4.

With the exception of the surface the fluctuations of the total abundance of cellulose-decomposing bacteria in sea water were less than could be accounted for by the sporadic distribution. The bacterial content of surface waters fluctuated widely, but there was no definite evidence of rhythmic seasonal variation. From these data it is doubtful whether the temperature has any influence on the total number of

cellulose-decomposing bacteria in sea water.

However, the distribution of certain definite species or groups of cellulose-decomposing bacteria fluctuated seasonally; kinds of cellulose-decomposing bacteria predominated in sea water distinctly varied seasonally.

The most abundant species contained in each sample of the sea water was as shown in Table 5. The species isolated from the enrichment culture medium inoculated with the water sample of the highest dilution was regarded as the most abundant species contained in that sample.

As will be shown later, *Vibrio purpureus*, *Vibrio purpureus* var. *albus*, and *Vibrio aquamarinus* which appeared in the sea predominantly during summer, were capable of growing at temperatures ranging from 20°C to 35°C. They decomposed cellulose rapidly at 30°C. On the other hand, *Vibrio marinoflavescens* and *Vibrio fulvus* which predominated in the sea during autumn, winter, and spring grew well at temperatures ranging from 20°C to 25°C but did not at temperatures higher than 30°C. It is, therefore, thought that the seasonal fluctuation of the species which predominated in the sea was correlated with their growth temperatures.

From the above mentioned observation it appears that the deterioration of cotton nettings exposed to sea water during summer months is primarily caused by the *Vibrio purpureus* group,* and that during winter months is mainly caused by the

Table 4. Seasonal distribution of cellulose-decomposing bacteria in sea water in Maizuru Bay.

Date of experiment	Approximate number of cellulose-decomposing bacteria in ml of sea water			Temperature of surface water °C	Salinity of surface water ‰
	Surface	2m in depth	4m in depth		
May 26, 1950	10	1	1	19	33
Jun. 5, 1950	10	1	1	19	31
Jun. 25, 1950	10	1	0	25	—
Jul. 5, 1950	10	1	1	24	28
Jul. 15, 1950	100	10	1	28	32
Jul. 25, 1950	100	—	1	27	31
Aug. 5, 1950	100	1	10	27	32
Aug. 15, 1950	10	10	1	29	34
Aug. 25, 1950	100	10	10	28	35
Sep. 5, 1950	100	10	10	24	23
Sep. 15, 1950	100	10	1	27	32
Oct. 25, 1950†	1	1	1	19	17
Nov. 26, 1950	10	10	10	12	—
Dec. 26, 1950	10	10	1	13	27
Feb. 10, 1954	100	10	10	10	—
Feb. 17, 1954	100	10	10	9	—
May 6, 1954	10	10	1	16	—

† Immediately after a heavy rain.

* *Vibrio purpureus*, *Vibrio purpureus* var. *albus*, *Vibrio aquamarinus*, *Vibrio gilvus*, *Vibrio albogilvus*, *Vibrio macerans*, and *Vibrio marinoliquefaciens* are included to the *Vibrio purpureus* group.

Table 5. Cellulose-decomposing bacteria which predominated in sea water in Maizuru Bay.

Date of collection	Most abundant species in sea water		
	Surface	2m in depth	4m in depth
May 26, 1950	*V. marinoflavescens*, *V. fulvus*, *V. aurantiacus*.	*V. marinoflavescens*, *V. fulvus*.	*V. marinoflavescens*, *V. fulvus*.
Jun. 5, 1950	*V. marinoflavescens*, *V. brevis*.	*V. marinoflavescens*, *V. fulvus*.	*V. fulvus*, *V. marinoflavescens*.
Jun. 25, 1950	*V. fulvus*, *V. marinoflavescens*.	*V. marinoflavescens*, *V. fulvus*, *V. purpureus*.	*V. fulvus*, *V. purpureus* var. *albus*.
Jul. 5, 1950	*V. purpureus*, *V. aquamarinus*, *V. fulvus*.	*V. marinoflavescens*, *V. fulvus*, *V. purpureus*.	*V. marinoflavescens*, *V. purpureus* var. *albus*.
Jul. 15, 1950	*V. aquamarinus*, *V. purpureus*, *V. macerans*.	*V. purpureus*, *V. aquamarinus*, *V. macerans*.	*V. purpureus*, *V. purpureus* var. *albus*, *V. aquamarinus*.
Jul. 25, 1950	*V. aquamarinus*, *V. purpureus*, *V. gilvus*.		*V. purpureus* var. *albus*, *V. purpureus*.
Aug. 5, 1950	*V. purpureus*, *V. aquamarinus*.	*V. purpureus*.	*V. purpureus* var. *albus*, *V. purpureus*.
Aug. 15, 1950	*V. aquamarinus*, *V. purpureus*.	*V. purpureus*.	*V. purpureus*.
Aug. 25, 1950	*V. purpureus*, *V. aquamarinus*.	*V. aquamarinus*, *V. purpureus*.	*V. purpureus*.
Sep. 5, 1950	*V. purpureus*, *V. aquamarinus*, *V. marinoflavescens*.	*V. aquamarinus*, *V. purpureus*, *V. marinoflavescens*.	*V. purpureus* var. *abus*, *V. purpureus*.
Sep. 15, 1950	*V. aquamarinus*, *V. purpureus*, *V. marinoflavescens*.	*V. aquamarinus*, *V. marinoflavescens*.	*V. marinoflavescens*, *V. purpureus* var. *albus*.
Oct. 25, 1950	*V. marinoflavescens*.	*V. marinoflavescens*.	*V. marinoflavescens*.
Nov. 26, 1950	*V. marinoflavescens*, *V. fulvus*.	*V. marinoflavescens*, *V. fulvus*.	*V. fulvus*, *V. marinoflavescens*.
Dec. 26, 1950	*V. marinoflavescens*.	*V. fulvus*, *V. marinoflavescens*.	*V. fulvus*, *V. marinoflavescens*.
Feb. 10, 1954	*V. fulvus*, *V. marinotypicus*.	*V. fulvus*, *V. simplex*.	*V. fulvus*, *V. marinoflavescens*.
Feb. 17, 1954	*V. fulvus*, *V. marinoflavescens*, *V. brevis*.	*V. fulvus*, *V. ferrugineus*.	*V. fulvus*, *V. marinotypicus*.
May 6, 1954	*V. fulvus*, *V. marinoflavescens*.	*V. euryhalis*, *V. fulvus*.	*V. fulvus*, *V. xanthus*.

Vibrio marinoflavescens group.*

As will be shown later, the *Vibrio purpureus* group was much greater with

* *Vibrio simplex*, *Vibrio ferrugineus*, *Vibrio brevis*, *Vibrio fulvus*, *Vibrio aurantiacus*, *Vibrio marinotypicus*, *Vibrio euryhalis*, *Vibrio marinoflavescens*, and *Vibrio xanthus* are included to the *Vibrio marinoflavescens* group.

respect to the cellulose-decomposing activity than the *Vibrio marinoflavescens* group at the optimum temperature for their growth. Therefore, seasonal fluctuation of the deterioration-rate of cotton fishing nets exposed to the sea may partly be correlated with the seasonal variation of the kinds of cellulose-decomposing organisms in the sea. Table 4 also showed that more cellulose-decomposing bacteria were nearly always found in surface waters than in deep waters. This may be attributable to the beneficial effects of the surface film of the sea water for marine microorganisms. The surface film may serve as mechanical support for microorganisms and miscellaneous particulate materials.

It has already been known that a large number of microorganisms were usually associated with the surface film of water when the sea was calm[28].

(2) Seasonal distribution of cellulose-decomposing bacteria belonging to genus *Cytophaga*.

As above described, cellulose-decomposing bacteria belonging to genus *Cytophaga* which may play an important role in the deterioration of cotton fishing nets exposed to the sea[33)34)] cannot be counted by the extinction dilution method as has been used for the enumeration of cellulose-decomposing true bacteria.

Therefore, for estimating the abundance of cellulose-decomposing bacteria belonging to genus *Cytophaga*, plate-count procedures were employed. The plate-count procedures employed were as follows: After 1 ml of the successively diluted* sea water or marine mud and about 8 ml of the sterilized sea water were uncontaminatedly placed in the sterilized petri dish, about 9 ml of the melted cellulose-agar medium was poured in the dish. After the agar had been solidified, the plate was incubated at 25°C.

The colonies of cellulose-decomposing cytophagas developed on the plates inoculated with appropriately diluted samples were counted. The colonies of the cytophagas were easily distinguished from those of the true bacteria by their external appearance.

Besides cytophagas, aerobic cellulose-decomposing true bacteria were also counted in the same time.

Samples of sea water and marine mud were collected almost monthly from different stations in Maizuru Bay from Nov. 1952 to Oct. 1953, using ZoBell's bacteriological water sampler[55] or Marukawa's grab.

The results obtained were as shown in Table 6, and might be summarized as follows:

1) Cellulose-decomposing cytophagas were widely distributed in water and bottom mud in Maizuru Bay. In such marine materials cytophagas constituted from 0 to 33.3% of the total number of aerobic cellulose-decomposing bacteria.

2) The abundance of cellulose-decomposing cytophagas in water and bottom

* As the dilution water, sterilized sea water was employed.

Table 6. Average number of aerobic cellulose-decomposing bacteria (belonging to *Cytophaga* and *Eubacteriales*) per ml of sea water or per g of bottom mud collected seasonally from different stations in Maizuru Bay.

Data of experiment	Station*	Sample inoculated	Depth of water in meters	Temperature of sample °C	Average number of aerobic cellulose-decomposing bacteria in 1 ml of sea water or in 1 g of mud		Ratio of *Cytophaga*/total aerobic cellulose-decomposing bacteria expressed as per cent
					Cytophaga	*Eubacteriales*	
Nov. 7, 1952	A	Sea water	Surface	18.0	4	25	13.8
Jan. 19, 1953	A	Sea water	Surface	6.0	5	21	19.2
Mar. 24, 1953	A	Sea water	Surface	10.5	3	27	10.0
Apr. 22, 1953	A	Sea water	Surface	13.0	8	30	21.1
May 15, 1953	A	Sea water	Surface	19.0	1	33	2.9
Jun. 16, 1953	A	Sea water**	Surface	23.0	0	20	0.0
Jun. 16, 1953	B	Sea water**	Surface	22.5	0	24	0.0
Jun. 16, 1953	B	Sea water**	7	18.4	0	7	0.0
Jun. 16, 1953	C	Sea water**	Surface	20.9	0	3	0.0
Jun. 16, 1953	C	Sea water**	7	18.5	0	2	0.0
Aug. 21, 1953	A	Sea water**	Surface	29.3	0	54	0.0
Aug. 21, 1953	B	Sea water**	Surface	29.3	0	62	0.0
Aug. 21, 1953	B	Sea water**	7	27.3	0	6	0.0
Aug. 21, 1953	B	Bottom mud	17	23.2	0	21	0.0
Aug. 21, 1953	C	Sea water*	Surface	29.4	0	14	0.0
Aug. 21, 1953	C	Sea water**	7	27.6	0	6	0.0
Aug. 21, 1953	C	Bottom mud	26	23.0	0	12	0.0
Oct. 8, 1953	A	Sea water	Surface	22.5	1	8	11.1
Oct. 8, 1953	B	Sea water	Surface	23.1	1	5	16.7
Oct. 8, 1953	B	Sea water	7	23.9	0	5	0.0
Oct. 8, 1953	B	Bottom mud	17	23.0	3	10	23.1
Oct. 8, 1953	C	Sea water	Surface	23.7	3	16	15.8
Oct. 8, 1953	C	Sea water	7	23.7	0	2	0.0
Oct. 8, 1953	C	Bottom mud	26	23.0	1	2	33.3

* Station A: Near the floating pier of Kyoto University.
Station B: Center of Maizuru Bay.
Station C: Near entrance to Maizuru Bay.

** Total numbers of plankton were extremely small in these water samples.

mud in Maizuru Bay fluctuated somewhat seasonally; in autumn, winter, and spring more or less living cells of the cytophagas were detected in the marine materials, but in summer such bacteria were never found.

The seasonal distribution of the cellulose-decomposing cytophagas in the sea seemed to be more closely correlated with the abundance of planktons than with the environmental temperature, because the abundance of planktons in the sea water was observed to be very small in June and August, 1953 when any living cells of the cytophagas was not found, and relatively hich counts of planktons were detected in the sea water in the other months.

Since planktons provide solid surfaces for the attachment of epiphytic bacteria, it is not surprising that the abundance of cytophagas is closely related to the abundance of planktons[53)54)]. The strong attachment property of cellulose-decomposing cytophagas may be more clearly explained by the data in Table 7.

The data given in Table 7 were obtained by the following procedures: A piece of 20/3 cotton cords (about 1 cm long) cut out from the fishing nets which had been submerged in the sea water in Maizuru Bay for 10 days, was vigorously ground down in 10 ml of sterilized sea water, to prepare the suspension of the bacterial cells which had attached to it. Then, after this suspension was appropriately diluted with sterilized sea water, 1 ml of it was poured together with about 8 ml of sterilized sea water and 9 ml of the melted cellulose agar medium into the sterilized petri-dish. After the agar had been solidified the plate was incubated at 25°C.

The colonies of cellulose-decomposing bacteria belonging to genus *Cytophaga* as well as to order *Eubacteriales* that developed on the plates were counted respectively.

The number of bacteria included in each group was expressed as a percent of the total number of aerobic cellulose-decomposing bacteria developed.

These experiments were carried out in different seasons in 1953.

Table 7. Relative numbers of aerobic cellulose-decomposing bacteria belonging to *Cytophaga* and *Eubacteriales* found in identical samples of cotton fishing nets which had been submerged in sea water near the floating pier of Kyoto University (station A) in Maizuru Bay for 10 days before sampling. The number of bacteria of each group was expressed as per cent of the total number of aerobic cellulose-decomposing bacteria developed.

Date of experiment	Temperature of sea water °C	Per cent of cytophagas	Per cent of true bacteria	Total aerobic cellulose-decomposing bacteria
Jan. 19, 1953	6.0	40.5	59.5	100
May 20, 1953	18.0	37.5	62.5	100
Aug. 21, 1953	29.3	22.2	77.8	100
Oct. 8, 1953	22.5	27.3	72.7	100

From Table 7 it was found that in the samples of fishing nets submerged in the sea cytophagas constituted from 22.2 to 40.5% of the total number of aerobic cellulose-decomposing bacteria. This percentage was more than twice as large as that found in the sea water where the sample of fishing nets had been immersed.

It is especially notable that an appreciable number of cytophagas were found on the nets immersed in the sea water* at station A from Aug. 11, 1953 to Aug. 21, 1953.

This result clearly indicates that cytophagas develop better on submerged surfaces such as fishing nets than in free-floating condition in the sea.

ZoBell[56] explained the beneficial effects of solid surfaces on the growth of bacteria in diluted nutrient solutions such as sea water as follows: Solid surfaces retard the diffusion of exoenzymes and partially digested food away from bacteria. Large molecules of organic matter must be converted into soluble substances by bacterial exoenzymes before the food can be assimilated by bacteria. Consequently

* Cytophagas were not found in sea water collected at station A on Aug. 21, 1953.

in a dilute nutrient solution such as sea water, bacterial cells which require comparatively large amounts of organic matters may not be able to digest and absorb enough nutrients in free floating conditions. However, when the bacterial cells and organic nutrients are juxtaposed on solid surfaces, the bacteria may more effectively absorb the food which has been rendered soluble by their exoenzymes. Bacteria in an anchored position are less influenced by molecular bombardment (with the resultant Brownian movement and diffusion) which would tend to separate them from their exoenzymes and hydrolyzates. Also, solid surfaces probably facilitate the orientation of exoenzymes in the most advantageous position, thereby increasing their stability and activity.

It is thought that the distribution of cellulose-decomposing bacteria belonging to genus *Cytophaga* is primarily influenced by the distribution of suspended solids, such as planktons, to which they preferentially attach.

Due to the tenacious attachment property and cellulose-decomposing ability, cytophagas may play an important role in the deterioration of fishing nets submerged in the sea.

2. Vertical and geographical distribution of aerobic cellulose-decomposing bacteria.

The present experiment was carried out in Hiroshima Bay and Maizuru Bay, in order to elucidate the effect of distance from land upon the horizontal and vertical distribution of cellulose-decomposing bacteria in the sea.

In Hiroshima Bay where the waters received a large amount of pulp mill drainage, the influence of the inflow of such drainage upon the vertical and geographical distribution of cellulose-decomposing bacteria in the sea was specially studied.

Stations chosen in the present experiment were as follows: Stations 3, 4, 5, 6, 7, 8, 9, and 10 were located in Hiroshima Bay at various distances (from 500 to 4,000 meters) from the draining mouth of the factory of the San-yō Pulp Mill Co. which produced a large amount of pulp mill waste (about 80,000 tons per day) that drained off into the Bay. Stations 11, 12, 13, 14, 15, and 16 were located in Hiroshima Bay at different distances (from 50 to 700 meters) from the point of entry of the drainage from the factory of the Aki Paper Manufacturing Co. Although the waste drainage from this factory was less in amount than that from the San-yō Pulp Mill Co., it contained cellulosic matter in higher concentration. Stations 1 and 2 chosen as the control stations in Hiroshima Bay were located in the vicinity of Atadajima Isl., where very little land drainage was found.

Stations 17, 18, 19, 20, 21, 22, 23, and 24 were located in Maizuru Bay at various distances (from −150 to 1,600 meters) off-shore from the mouth of the Isatsu River. Station 25 was near the entrance to Maizuru Bay.

The observations on the distribution of cellulose-decomposing bacteria in Hiro-

Table 8. Approximate number of aerobic cellulose-decomposing bacteria and some chemical and physical properties of sea water and bottom mud collected at different distances from land in the vicinity of Atada jima Island, in Hiroshima Bay. (May 20, 1954)

Station number	Distance from land (m)	Depth of the overlying water (m)	Depth of water collected (m)	Water temperature (°C)	Temperature of mud (°C)	Chlorinity of water (‰)	C.O.D. of mud (O_2 consumed mg/g dry mud)	Loss on ignition (% of dry mud)	Aerobic cellulose-decomposing bacteria/1 ml or 1 g of sample
1	500	29.5	0	17.9		16.28			1
			12	15.8		17.97			1
					15.0		7.45	11.86	10
2	2,000	23.0	0	17.8		16.25			0
			12	15.7		17.98			1
					15.3		5.84	11.41	10

Table 9. Approximate number of aerobic cellulose-decomposing bacteria and some chemical or physical properties of sea water and bottom mud collected at different distances from the mouth of the main drainage of the factory of San-yō Pulp Mill Company in Hiroshima Bay. (May 18, 1954)

Station number	Distance from the draining mouth (m)	Depth of the overlying water (m)	Depth of water collected (m)	Water temperature (°C)	Temperature of mud (°C)	Chlorinity of water (‰)	C.O.D. of mud (O_2 consumed mg/g dry mud)	Loss on ignition (% of dry mud)	Aerobic cellulose-decomposing bacteria in 1 ml or 1 g of sample
3	500	3.0	0	20.0		14.55			1
			2.0	17.4		16.14			100
					17.2		5.17	4.30	10,000
4	1,000	12.0	0	19.8		15.01			1
			11.0	15.4		18.18			0
					17.2		13.86	10.51	100
5	1,500	15.0	0	20.2		15.01			10
			12.0	15.3		18.14			1
					15.0		19.66	11.45	100
6	2,000	24.0	0	19.4		16.61			0
			12.0	15.9		18.09			0
					14.6		15.89	11.91	1
7	3,000	27.0	0	18.0		15.79			0
			12.0	15.9		18.09			0
					14.6		11.85	12.54	10
8	4,000	32.0	0	17.6		17.50			0
			12.0	16.2		18.09			0
					14.8		7.67	12.21	10
9	1,500	14.5	0	18.1		13.88			10
			13.0	15.2		18.35			1
					14.8		18.34	7.95	100
10	1,000	12.0	0	21.0		14.23			10
			10.0	16.0		—			1
					15.5		19.49	9.44	100

shima Bay were carried out in May, 1954, and those in Maizuru Bay were carried out in June, 1954 and in September, 1954.

At the aforementioned stations, sea water at different depths, and bottom mud were collected and examined, by means of the extinction dilution method, for finding the total population of aerobic cellulose-decomposing bacteria.

In addition to the bacteriological examination, the chlorinity of the water and the amounts of C. O. D. and of ignition loss of mud were determined. In some cases the amount of free H_2S in mud was determined. And also in all cases the temperature was recorded.

The results obtained were as shown in Tables 8, 9, 10, 11, and 12.

As illustrated in these Tables, the population of cellulose-decomposing bacteria was always larger in bottom deposit than in the overlying water, in which a marked stratification of the organisms was never observed.

It is thought that in such regions water movements tend to prevent the bacterial stratification in the overlying water.

In the vicinity of Atadajima Isl. (stations 1 and 2) where very little land drainage was found, the count of aerobic cellulose-decomposing bacteria was almost

Table 10. Approximate number of aerobic cellulose-decomposing bacteria and some chemical or physical properties of sea water and bottom mud collected at different distances from the mouth of the main drainage of the factory of Aki Paper-Manufacturing Company, in Hiroshima Bay. (May 20, 1954)

Station number	Distance from the draining mouth (m)	Depth of the overlying water (m)	Depth of water collected (m)	Water temperature (°C)	Temperature of mud (°C)	Chlorinity of water (‰)	C.O.D. of mud (O_2 consumed mg/g dry mud)	Loss on ignition (%)	Free H_2S content of mud (mg/g dry mud)	Aerobic cellulose-decomposing bacteria in 1 ml or 1 g of sample
11	50	3.5	0	18.1		15.43				100
			2.5	18.2		17.24				10
					17.1		529.88	57.31	3.405	100
12	100	10.0	0	18.5		15.60				10
			9.0	16.0		18.08				10
					15.7		150.31	21.99	0.487	100
13	200	11.0	0	18.0		15.19				10
			10.0	15.4		18.10				100
					15.3		27.26	13.20	0.065	100,000
14	400	12.0	0	18.0		15.72				10
			11.0	15.5		18.33				100
					15.4		16.88	9.74	0.053	10,000
15	700	14.0	0	18.0		15.71				10
			12.0	16.0		18.08				10
					15.3		11.06	8.85	0.026	1,000
16	200	7.0	0	18.0		15.97				100
			6.0	16.0		17.60				1
					15.4		71.83	16.74	0.174	1,000

Table 11. Approximate number of aerobic cellulose-decomposing bacteria and some chemical or physical properties of sea water and bottom mud collected at different distances from the mouth of the Isatsu River in Maizuru Bay (June 18, 1954).

Station number	Distance from the river mouth (m)	Depth of the overlying water (m)	Depth of water collected (m)	Water temperature (°C)	Temperature of mud (°C)	Chlorinity of water (‰)	C.O.D. of mud (O_2 consumed mg/g dry mud)	Loss on ignition (% of dry mud)	Aerobic cellulose-decomposing bacteria in 1 ml or 1 g of sample
			0	21.2		14.52			0
17	1,600	13.0	12.0	18.4		18.87			0
					18.2		22.6	9.15	1
			0	22.4		13.92			1
18	1,100	12.0	11.0	19.7		18.15			10
					18.2		37.2	10.55	100
			0	22.7		13.25			10
19	600	9.0	8.0	19.0		18.52			0
					18.2		44.0	10.54	100
			0	22.2		12.85			1
20	150	3.5	2.5	19.5		18.35			1
					19.5		56.0	11.01	1
			0	23.4		4.42			10
21*	−150*	3.0	2.0	20.0		17.52			100
					19.9		41.5	11.25	100
			0	21.6		14.62			10
22	450	8.3	7.0	18.8		18.35			1
					18.5		29.2	13.82	10
			0	22.8		14.72			0
23	800	10.0	9.0	18.4		18.82			0
					18.8		36.8	10.36	1
			0	22.3		14.65			10
24	500	8.2	7.0	18.7		18.72			1
					18.6		29.8	9.54	10

* In the river.

constant (0–1 per 1 ml of water and 10 per 1 g of mud) regardless of the distance from land or the depth of water. In such regions of the sea, it seems that neither distance from land nor depth of the overlying water had any direct influence upon the abundance of cellulose-decomposing bacteria in the mud or in the overlying water.

On the contrary, in the regions of the sea receiving industrial drainage from pulp mills (stations 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, and 16), the abundance of aerobic cellulose-decomposing bacteria in the bottom mud generally increased with the proximity to the draining mouths (except in the cases of stations 11 and 12), although such a phenomenon was not recognized clearly in the overlying water.

The data in Tables 8, 9, and 10 show that in Hiroshima Bay the population of aerobic cellulose-decomposing bacteria in the bottom mud was correlated to a

Table 12. Approximate number of aerobic cellulose-decomposing bacteria and some chemical or physical properties of sea water and bottom mud collected at different distances from the mouth of the Isatsu River in Maizuru Bay. (Sept. 29, 1954)

Station number	Distance from the river mouth (m)	Depth of the overlying water (m)	Depth of water collected (m)	Water temperature (°C)	Temperature of mud (°C)	Chlorinity of water (‰)	C.O.D. of mud (O_2 consumed mg/g dry mud)	Loss on ignition (% of dry mud)	Aerobic cellulose-decomposing bacteria in 1 ml or 1 g of sample
			0	23.2		15.50			10
17	1,600	13.0	10.0	23.2		18.05			1
					23.0		27.0	11.30	10
			0	22.8		15.33			1
18	1,100	11.5	10.0	23.2		18.15			10
					23.1		34.6	11.26	100
			0	22.2		15.05			10
19	600	8.0	7.0	23.6		18.15			100
					23.2		23.1	11.26	1,000
			0	23.3		15.75			10
20	150	4.0	3.0	23.8		16.88			10
					23.6		25.9	10.00	100
			0	22.7		8.85			100
21*	-150*	3.0	2.0	23.6		17.05			10
					23.8		35.0	9.83	1,000
			0	22.9		15.65			100
22	450	8.3	7.0	23.7		17.73			10
					23.7		32.4	11.63	100
			0	22.6		15.33			1
23	800	8.0	7.0	23.7		17.95			10
					23.2		34.0	9.99	100
			0	22.5		15.15			10
25**	8,000	25.0	10.0	23.3		18.25			10
					22.7		30.2	11.03	100

* In the river.
** Entrance to Maizuru Bay.

certain extent with the availability of organic matter contained but was not correlated with the distance from the land or with the depth of the overlying water.

The number of aerobic cellulose-decomposing bacteria in relation to C. O. D. and ignition loss of the bottom mud was illustrated in Fig. 2. It is generally held that the values of C. O. D. show the contents of easily decomposable organic matter and those of ignition loss show the contents of total organic matter including the relatively refractory substances such as "marine humas".[55]

From Fig. 2, therefore, it is suggested that the abundance of aerobic cellulose-decomposing bacteria in the bottom mud in Hiroshima Bay was more dependent upon the quality -decomposability- of the organic matter than upon the total quantity of the organic matter, and that the refractory organic matter, being designed as

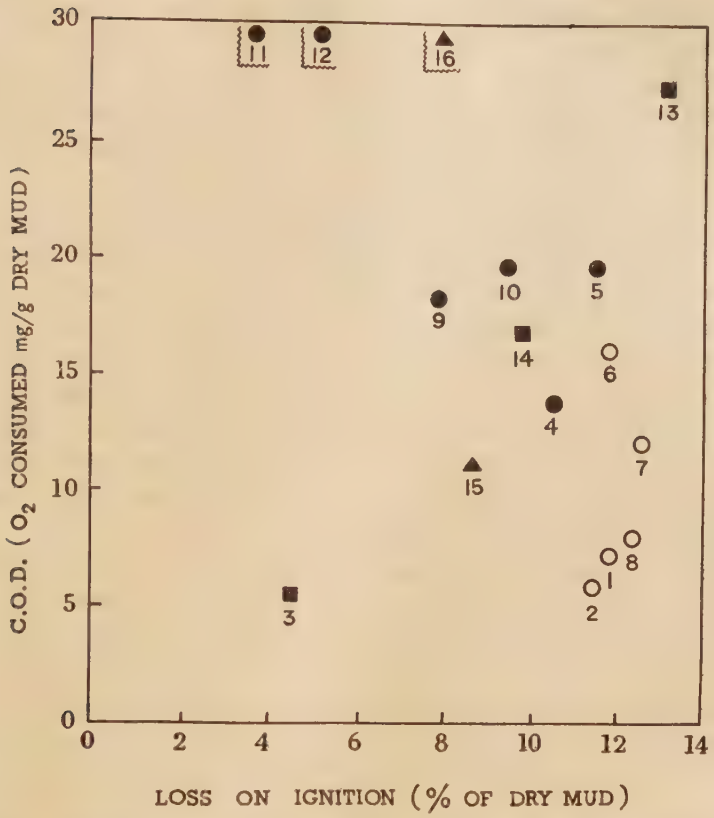


Fig. 2. Relation between C.O.D. and loss on ignition of the bottom mud samples collected at various stations in Hiroshima Bay. ○ : 1–10 cells of aerobic cellulose-decomposing bacteria were contained in 1g of mud (wet basis); ● : 100 cells of cellulose-decomposers were contained in 1g of mud (wet basis); ▲ : 1,000 cells of cellulose-decomposers were contained in 1g of mud (wet basis); ■ : more that 10,000 cells of cellulose decomposers were contained in 1g of mud (wet basis).

"marine humas" by WAKSMAN,[55–57] was hardly utilized by such bacteria.

In the unpolluted regions of the sea the greater part of organic matter contained in the bottom mud seems to consist of material which is fairly refractory to bacterial decomposition and, therefore, the abundance of cellulose-decomposing bacteria in such a bottom mud may be comparatively small.

However, in the regions of the sea receiving a considerable amount of drainage containing organic matter available to cellulose-decomposing bacteria in high concentration (such as pulp mill drainage), the activity of cellulose-decomposing bacteria in the bottom mud may be vigorously accelerated, because such organic matter is continuously brought onto the sea floor.

Small abundance of aerobic cellulose-decomposing bacteria was found, in spite

of the presence of a large amount of cellulosic matter, in mud samples collected from the stations 11 and 12. This seemed to be attributed to an anaerobic condition caused by the presence of high concentration of H_2S which was produced by the sulfate-reducing bacteria.[58] And also this may be understood from the fact that the kinds of microorganisms existing in bottom deposit are influenced by the oxygen tension.[59]

In Maizuru Bay the abundance of aerobic cellulose-decomposing bacteria not only in sea water but also in bottom mud fluctuated topographically, although no regularity was found in the horizontal distribution of such bacteria. In this bay neither distance from the mouth of the Isatsu River nor the quality of organic matter contained in the bottom mud had any direct influence upon the population of these bacteria in the mud. The bottom mud in Maizuru Bay generally contained a relatively large quantity of easily decomposable organic matter. It is, therefore, thought that in this bay the distribution of aerobic cellulose-decomposing bacteria in the bottom mud was influenced by other complex factors rather than by the quality of organic matter contained in the mud.

Although it is difficult to appraise quantitatively each of the numerous inter-related factors which influence the distribution of these bacteria in the sea, from all the data available it appears established that the population of these organisms was always larger in bottom deposits than in overlying water, in which the marked stratification of these bacteria was not found; that neither distance from land nor depth of overlying water had any direct influence upon the population of these bacteria in sea water and in bottom deposit; that the horizontal distribution of these bacteria in bottom mud was correlated to a certain extent with the decomposability but not the total quantity of organic matter contained in the mud, provided that the organic matter is a little in the decomposability; that the smaller abundance of these bacteria was found in mud which contained a large amount of H_2S.

IV. Aerobic cellulose-decomposing bacteria occurring on cotton fishing nets.

1. Changes in flora of aerobic cellulose-decomposing bacteria occurring on cotton fishing nets during immersion in sea water.

In order to elucidate what kinds of bacteria were mainly responsible for the deterioration of cotton fishing nets during immersion in sea water, changes in flora of cellulose-decomposing bacteria occurring on the nets during immersion were observed.

The samples of fishing nets used for the present experiment were submerged in sea water at the station near the floating pier of Kyoto University in Maizuru Bay. After immersion for the desired periods, the relative number of cells of two main groups of aerobic cellulose-decomposing bacteria (cytophagas and true bacteria)

occurring on the nets were counted by means of the plating procedure.

The results obtained were given in Table 13.

Table 13. Relative number of aerobic cellulose-decomposing bacteria belonging to *Cytophaga* and *Eubacteriales* found on the fishing nets which had been submerged in sea water at the station near the floating pier of Kyoto University in Maizuru Bay for desired periods. The number of bacteria of each group was expressed as per cent of the total number of aerobic cellulose-decomposing bacteria developed.

Immersion time in days	Percentage of two main groups of marine aerobic cellulose-decomposing bacteria.	
	Cytophagas	True bacteria
0*	0	0
9	16.0	84.0
50	16.6	83.4
60	33.3	66.7
75	20.0	80.0
88	13.8	86.2

* One hundred and ten cells of aerobic cellulose-decomposing bacteria (6 of which belonged to *Cytophaga* and 104 of which belonged to *Eubacteriales*) were contained in 1 ml of sea water in which the cotton fishing nets were submerged (June 7, 1954).

This result suggests that cellulose-decomposing bacteria belonging to genus *Cytophaga* developed more preferentially on submerged surfaces of fishing nets than in free-floating condition in the sea. After 9 days' immersion, the proportion between the number of cytophagas and that of true bacteria remained practically constant throughout a long period of immersion, although the absolute number of both groups might increase respectively in the course of the immersion period.

Throughout the period of immersion, however, aerobic cellulose-decomposing true bacteria always predominated over cellulose-decomposing cytophagas in number, on the nets.

Changes in the tensile strength and in the molecular weight of cotton yarn of the fishing nets were observed at the same station by KISHIMOTO[60] of our Institute, at approximately the same time when this bacteriological test was being carried out and the results obtained by him were as shown in Fig. 3.

2. Cellulose-decomposing bacteria responsible for the deterioration of cotton fishing nets during storage after fishing.

Besides deteriorating during immersion in the sea, cotton fishing nets are known to rot during storage after fishing.

The present experiment, therefore, was undertaken to determine the organisms which were responsible for the deterioration of cotton fishing nets during storage after immersion in the sea.

The samples were taken up from the sea and then immediately stored both in

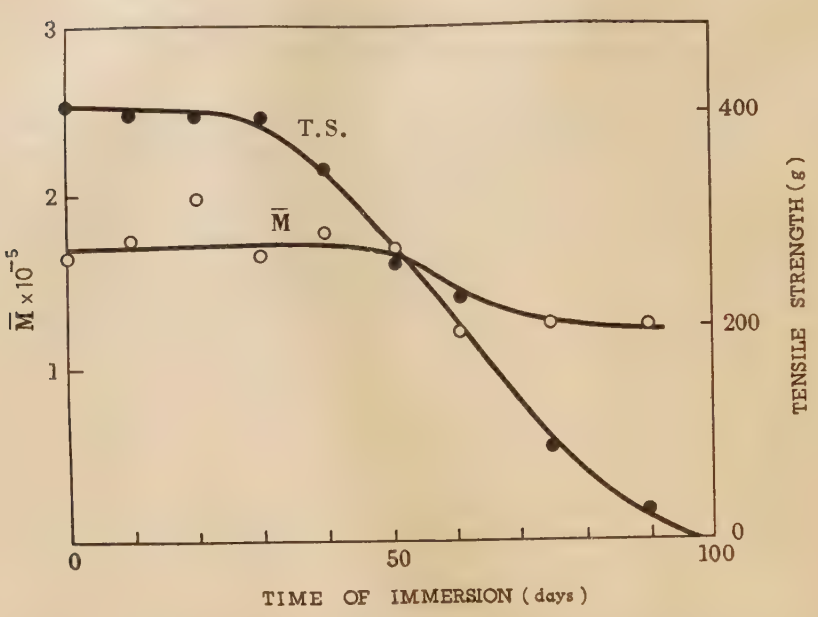


Fig. 3. Averaged molecular weight and tensile strength of cotton yarn as functions of time of immersion. ○ : Molecular weight; ● : Tensile strength. (From KISHIMOTO[60])

a wet as well as in a dry condition in order to demonstrate the influence of moisture upon the bacterial flora incidentally. For this experiment, fishing nets immersed for periods of 10 to 30 days in sea water at the station near the floating pier of Kyoto University in Maizuru Bay were employed as samples. To maintain them

Table 14. Comparison of number of living cells of two main groups of aerobic cellulose-decomposing bacteria occurring on cotton fishing nets which had been stored for desired periods, under wet conditions, after 30 days' immersion (from Jan. 11, 1954) in sea water at the station near the floating pier of Kyoto University in Maizuru Bay. The number of bacteria of each group was expressed as per cent of the total number of marine aerobic cellulose-decomposing bacteria developed.

(i) Storage temperature: 9-15°C (room temperature)

Storage time in days	Water content of the nets (%)	Percentage of two main groups of marine aerobic cellulose-decomposing bacteria	
		Cytophagas	True bacteria
0	62.3	25.9	74.1
7	59.2	24.2	75.8
14	59.0	28.0	72.0
21	61.5	76.2	23.8
28	62.0	65.0	35.0
34	59.8	69.2	30.8
53	59.5	60.0	40.5

(ii) Storage temperature: 30°C

Storage time in days	Water content of the nets (%)	Percentage of two main groups of marine aerobic cellulose-decomposing bactera	
		Cytophagas	True bacteria
0	61.5	25.0	75.0
7	60.5	94.8	5.2
14*	62.3	79.3	20.7
21*	64.1	96.8	3.2
27*	61.0	94.4	5.6
46*	61.0	—**	—**

* At the bottom of the bottle sulfate reducing bacteria developed and a large amount of H_2S were produced.

** No aerobic cellulose-decomposing bacteria developed.

Table 15. Comparison of numbers of survived cells of two main groups of marine aerobic cellulose-decomposing bacteria occurring on the cotton fishing nets which had been stored for desired periods, under dry conditions, after 10 days' immersion (from Jan. 9, 1953 to Jan. 19,1953) in sea water at the station near the floating pier of Kyoto University in Maizuru Bay. The number of bacteria of each group was expressed as per cent of the total number of marine aerobic cellulose-decomposing bacteria developed.

Storage time in days	Water content of the nets (%)	Percentage of two main groups of marine aerobic cellulose-decomposing bacteria	
		Cytophagas	True bacteria
0	48.1	40.5	59.5
4	6.8	58.3	41.7
14	8.4	66.4	33.6
22	7.5	80.0	20.0
29	11.0	80.0	20.0
48	8.3	75.0	25.0

Twenty nine cells of aerobic cellulose-decomposing bacteria (8 of which belonged to *Cytophaga*, and 21 of which belonged to *Eubacteriales*) were contained per 1 ml of sea water in which the fishing nets were submerged (Jan. 19, 1953).

in a wet condition, the samples were placed in a glass bottle which was so equiped that the saturated humidity might constantly be maintained in it. And also in the case of dry condition, the samples were placed on the floor, as they were. After having been left for desired period, the samples were examined with respect to the relative number of cells of two main groups of aerobic cellulose-decomposing bacteria (cytophagas and true bacteria) which survived, by means of the plating procedure.

Changes in the quantitative relation between the two main groups present on the cotton fishing nets during storage were as shown in Tables 14 (under wet condition) and 15 (under dry condition).

The data in Tables 14 and 15 show that living cells of marine aerobic cellulose-

decomposing bacteria present on the cotton fishing nets after immersion in the sea survived or developed for a considerably long period during storage whether the nets were dry or wet.

However, the proportion of aerobic cellulose decomposing bacteria belonging to *Eubacteriales* or to *Cytophaga* to the total aerobic cellulose-decomposing bacteria occurring on the nets was not constant throughout the period of storage; the relative number of living cells of cytophagas increased progressively with the time of storage. This increase was especially noticeable at 30°C on the wet net.

This result suggests that the marine cellulose-decomposing cytophagas were more resistant or more adaptable to such storage conditions than marine aerobic cellulose-decomposing true bacteria. The fact that the true bacteria rapidly decreased in the relative number of living cells at 30°C under wet condition, might be caused by their higher sensitivity to the free H_2S produced by marine sulfate reducers in the bottle.

From the above mentioned it is suggested that marine cellulose-decomposing cytophagas may play an important role in the deterioration of cotton fishing nets during storage after fishing.

And again, from the above mentioned facts it may be considered that the marine cellulose-decomposing cytophagas play a more important role than the cellulose-decomposing true bacteria in the deterioration of fishing nets during the period of storage after fishing or during the period of use again after storage, although the latter bacteria may play a more important role than the former in the deterioration of the nets newly immersed in the sea.

V. Some of the environmental factors influencing the growth and the activity of marine aerobic cellulose-decomposing bacteria.

It is probable that the activity of marine aerobic cellulose-decomposing bacteria may be greatly influenced by various environmental factors.

In this chapter the effects of temperature, pH value, and salinity which seem to influence primarily the activity of marine cellulose-decomposing bacteria were studied.

1. Temperature

The growth of each of the marine aerobic cellulose-decomposing bacteria in the liquid mineral medium (prepared with sea water) with filter paper partly immersed was observed at various temperatures.

The results obtained were given in Table 16.

The data in Table 16 reveal that there were distinct differences, with respect to the temperature ranges for their growth, between the species predominating in summer and those predominating in winter.

In general, the species predominating in summer developed preferentially at

Table. 16. The growth of marine aerobic cellulose-decomposing bacteria at various temperatures.

Organism / Incubation temperature °C	15	17	20	25	30	35	40
Pseudomonas marinocellulosae	+	++	+++	+++	+	−	−
Vibrio aquamarinus	−	−	+	+++	+++	++	−
Vibrio purpureus	±	+	++	+++	+++	++	−
Vibrio purpureus var. *albus*	±	+	++	++	+++	++	−
Vibrio gilvus	−	−	−	++	+++	++	−
Vibrio albogilvus	−	−	+	+++	++	++	−
Vibrio macerans	−	−	+	++	+++	++	−
Vibrio marinoliquefaciens	−	−	++	++	+++	++	−
Vibrio simplex	++	++	+++	+++	+	−	−
Vibrio ferrugineus	++	++	+++	+++	++	−	−
Vibrio brevis	++	++	+++	+++	++	−	−
Vibrio fulvus	+	++	+++	+++	+	−	−
Vibrio aurantiacus	−	−	+++	+++	++	−	−
Vibrio marinotypicus	−	−	+++	+++	++	−	−
Vibrio euryhalis	−	−	++	+++	++	+	−
Vibrio marinoflavescens	+	++	+++	+++	++	−	−
Vibrio xanthus	++	+++	+++	+++	++	−	−
Cytophaga haloflava	+	++	+++	+++	++	−	−
Cytophaga haloflava var. *nonreductans*	++	++	++	+++	++	−	−
Cytophaga rosea	−	−	+	+++	++	−	−

− = no growth, +++ = maximum growth.

35°C, whereas the development of the species predominating in winter ceased at a temperature of 35°C.

From this result it appears that the temperature is one of the important factors which influence the kinds and the activities of these bacteria in the sea, especially on the surfaces of submerged cotton fishing nets which may provide not only sufficient energy sources but adherent-surfaces for such organisms.

The temperature tolerance of the marine aerobic cellulose-decopmosing bacteria was determined by the following procedures: The organisms grown for 5 days at 25°C in the sea water medium (cellobiose, 1.0 g; $NaNO_3$, 0.5 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; filtered sea water, 1000 ml, pH 7.2) were held at 40,50, or 60°C for 5, 10, 20, 30, or 40 minutes. These heated cultures were respectively inoculated in amount of 5 loops to 10 ml of the above mentioned medium and incubated at 25°C. After 5 days' incubation, it was determined by visual observation whether the growth occurred or not.

The results obtained were as shown in Table 17.

Table 17. Thermal death points of the marine aerobic cellulose-decomposing bacteria.

Species \ Exposure temperature °C / Exposure time in minutes	40					50					60				
	5	10	20	30	40	5	10	20	30	40	5	10	20	30	40
Pseudomonas marinocellulosae	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Vibrio aquamarinus	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
Vibrio purpureus	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Vibrio purpureus var. *albus*	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Vibrio gilvus	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−
Vibrio albogilvus	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Vibrio macerans	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−
Vibrio marinoliquefaciens	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Vibrio simplex	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Vibrio ferrugineus	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Vibrio brevis	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
Vibrio fulvus	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Vibrio aurantiacus	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−
Vibrio marinotypicus	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
Vibrio euryhalis	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−
Vibrio marinoflavescens	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
Vibrio xanthus	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Cytophaga haloflava	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Cytophaga haloflava var. *nonreductans*	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−
Cytophaga rosea	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−

− = no growth, + = normal growth.

The data in Table 17 show that the majority of the marine aerobic cellulose-decomposing bacteria survived the exposure to a temperature of 40°C for 40 minutes, but died when exposed to 50°C for 5 to 20 minutes. Comparing these results with the data concerning the heat resistance of general marine heterotrophs reported by ZoBell and Conn[48] reveals that the average thermal death point of general marine heterotrophs is considerably lower than that of the marine aerobic cellulose-decomposing bacteria (Table 18).

2. pH values

In order to elucidate the effect of the pH upon the growth of marine aerobic cellulose-decomposing bacteria, the growth of each of these organisms in the sea water mineral media (with filter paper partly immersed) having pH values ranging from 4.0 to 9.0 was observed at 25°C.

Table 18. The percentage survival of bacteria in sea water and marine mud at various temperatures. (From ZoBell and Conn[48])

Samples held 10 minutes at	20°C	30°C	40°C	50°C	80°C	100°C
Per cent survival in sea water	100	81.3	21.9	6.8	3.0	< 1
Per cent survival in marine mud	100	68.5	18.3	10.3	5.2	< 1

Table 19. The effect of the pH upon the growth of marine aerobic cellulose-decomposing bacteria.

The pH value of the medium	Organism	*Pseudomonas marinocellulosae*	*Vibrio aquamarinus*	*Vibrio purpureus*	*Vibrio purpureus* var. *albus*	*Vibrio gilvus*	*Vibrio albogilvus*	*Vibrio macerans*	*Vibrio marinoliquefaciens*	*Vibrio simplex*	*Vibrio ferrugineus*	*Vibrio brevis*	*Vibrio fulvus*	*Vibrio aurantiacus*	*Vibrio marinotypicus*	*Vibrio euryhalis*	*Vibrio marinoflavescens*	*Vibrio xanthus*	*Cytophaga haloflava*	*Cytophaga haloflava* var. *nonreductans*	*Cytophaga rosea*
	4.0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
	5.0	−	−	−	−	−	±	−	−	−	−	−	−	−	−	++	−	−	−	−	−
	6.0	++	++	++	++	−	++	+	++	++	++	++	++	++	++	++	++	++	−	++	−
	7.0	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	++	+++	+++	+++	+++	+++	++	+++	+++	+++
	8.0	+++	++	+++	+++	+++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++
	9.0	++	++	++	++	+++	−	−	−	++	++	++	++	++	++	++	++	++	−	++	++

− = no growth, +++ = maximum growth.

The results obtained were illustrated in Table 19. This table shows that nearly

all the marine aerobic cellulose-decomposing bacteria tested grew well in the media having pH values ranging from 6.0 to 9.0.

The pH encountered in the sea ranges from 7.5 to 8.5. When in equilibrium with the CO_2 of the atmosphere, the pH of sea water ranges from 8.1 to 8.3. In the euphotic zone photosynthetic plants may reduce the CO_2 content of the water until the pH reaches 8.3 to 8.5 during the hours of intense sunlight. Below the euphotic zone the pH generally decreases with depth to a minimum of 7.5 at depths exceeding 1,000 meters. Therefore, it is generally thought that in the sea water the pH cannot be a limiting factor for the development and the activity of the marine aerobic cellulose-decomposing bacteria.

3. Salinity

The effect of the salinity upon the growth of marine aerobic cellulose-decomposing bacteria was studied by observing the growth of each of these organisms in the liquid mineral media (with filter paper partly immersed) prepared with NaCl solu-

Table 20. Development of the marine aerobic cellulose-decomposing bacteria in the media prepared with sea water or NaCl solutions containing NaCl in different concentrations.

NaCl content of the medium (%)	*Pseudomonas marinocellulosae*	*Vibrio aquamarinus*	*Vibrio purpureus*	*Vibrio purpureus* var. *albus*	*Vibrio gilvus*	*Vibrio albogilvus*	*Vibrio macerans*	*Vibrio marinoliquefaciens*	*Vibrio simplex*	*Vibrio ferrugineus*	*Vibrio brevis*	*Vibrio fulvus*	*Vibrio aurantiacus*	*Vibrio marinotypicus*	*Vibrio eurybalis*	*Vibrio marinoflavescens*	*Vibrio xanthus*	*Cytophaga haloflava*	*Cytophaga haloflava* var. *nonreductans*	*Cytophaga rosea*
0	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
0.5	−	−	±	±	−	−	−	+	−	−	−	+	−	−	+	+	±	−	−	−
1.0	−	++	+	+	++	−	+	++	−	++	−	+	++	++	++	++	−	+	−	−
3.0	−	+++	+++	+++	+++	−	+++	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
5.0	−	−	±	±	−	−	++	++	+	++	++	+	+	+	++	+	++	+	+	−
10.0	−	−	±	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Sea water	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

* − = no growth, +++ = maximum growth.

tions containing NaCl in different concentrations or with natural sea water at 25°C.

The results obtained were given in Table 20.

Of the media which were prepared with NaCl solutions containing NaCl in different concentrations, the medium containing 3% of NaCl was most suitable for the development of the marine aerobic cellulose-decomposing bacteria. These bacteria, excepting *Vibrio euryhalis*, could develop neither in the medium prepared with fresh water nor in the medium prepared with a 10% NaCl solution. And also of all of the bacteria tested, only a few species developed in the media prepared with a 0.5, 1.0, or 5.0% NaCl solution.

These results suggest that while these bacteria may be characterized as being halophilic or salt-requiring, they are far less so than are bacteria found in salted fish, salt lakes, and limans.

The preference of the marine aerobic cellulose-decomposing bacteria for sea water over NaCl solution isotonic with sea water was also clearly illustrated by the data in Table 20, *viz.*, all the bacteria examined grew more luxuriantly in the media prepared with natural sea water than in those prepared with NaCl solutions. Especially, *Pseudomonas marino cellulosae* and *Vibrio albogilvus* grew exclusively in the media prepared with natural sea water. These results may suggest that natural sea water contained certain accessory constituents which promoted the growth of these organisms.

According to SVERDRUP et al.[40] the salinity of deep or bottom water of the oceans varies within narrow limits, approximately 34.6 to 35‰. Unless diluted by heavy rainfall, melting ice, or rivers, the salinity of surface sea water generally ranges from 33 to 37‰. Therefore, it is generally thought that in the sea water the salinity hardly limits the development and the activity of the marine aerobic cellulose-decomposing bacteria.

VI. Some of the physiological and biochemical characteristics of the marine aerobic cellulose-decomposing bacteria.

General characteristics of the marine aerobic cellulose-decomposing bacteria were separately described in chapter III. In this chapter some of their characteristics which may be important in considering the microbiological deterioration of cotton fishing nets were studied.

1. Organic compounds utilized by the marine aerobic cellulose-decomposing bacteria as the carbon sources.

Using a balanced mineral solution ($NaNO_3$, 0.5 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; filtered sea water, 1000 ml, pH 7.2) as the basal medium, the utilization of various organic compounds as the carbon source by the marine aerobic cellulose-decomposing bacteria was studied at 25°C.

The development of the marine aerobic cellulose-decomposing bacteria in the

Table 21. The utilization of various organic compounds by marine aerobic cellulose-decomposing bacteria as a sole source of carbon.

Species \ Organic compound	Xylose	Arabinose	Rhamnose	Glucose	Fructose	Galactose	Mannose	Cellobiose	Maltose	Lactose	Trehalose	Sucrose	Raffinose	Cellulose	Starch	Inulin	Agar	Glycerin	Erythritol	Mannitol	Inositol	Dulcitol	Salicin	Ca-lactate	Ca-gluconate	Na-alginate
Pseudomonas marinocellulosae	+	−	−	+	+	+	−	+	+	−	−	+	−	+	+	−	−	+	−	+	−	−	−	−	−	+
Vibrio aquamarinus	+	+	−	+	−	+	+	+	+	+	−	+	−	+	+	−	+	−	−	−	−	−	+	−	−	+
Vibrio purpureus	−	−	−	+	+	+	+	+	−	−	−	+	−	+	+	−	+	−	−	−	−	−	+	−	−	+
Vibrio purpureus var. *albus*	−	−	−	+	+	+	−	+	−	−	−	+	−	+	+	−	+	−	−	−	−	−	+	−	−	+
Vibrio gilvus	−	+	−	+	−	+	+	+	+	+	−	+	−	+	+	−	−	−	−	−	−	−	+	−	−	−
Vibrio albogilvus	+	+	−	+	+	+	+	+	+	+	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−
Vibrio macerans	+	+	−	+	−	+	−	+	+	+	+	+	+	+	+	+	−	+	−	−	+	−	+	−	+	−
Vibrio marinoliquefaciens	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	−	+	+	+	+
Vibrio simplex	+	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Vibrio ferrugineus	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Vibrio brevis	−	+	−	+	+	+	+	+	−	−	+	−	−	+	−	−	−	−	−	+	+	−	−	−	+	−
Vibrio fulvus	+	+	−	+	+	+	+	+	−	+	−	+	+	+	−	−	−	+	−	+	−	−	+	+	+	−
Vibrio aurantiacus	+	−	−	+	−	−	+	+	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Vibrio marinotypicus	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−	−	+	+	−	+	
Vibrio euryhalis	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	−	+	+	+	+	+	+	+	−	
Vibrio marinoflavescens	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	−	
Vibrio xanthus	+	−	−	+	−	−	−	+	−	−	+	+	−	+	+	−	−	+	−	−	−	−	−	−	−	−
Cytophaga haloflava	−	−	−	+	−	−	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Cytophaga haloflava var. *nonreductans*	−	−	−	+	−	−	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Cytophaga rosea	−	−	−	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−

− = no growth, + = growth.

media containing a variety of organic compounds as the sole carbon source was as shown in Table 21.

The results obtained indicate that the species varied widely in ability to utilize organic compounds as a carbon source.

Among the organic compounds tested, only a specialized few compounds such as cellulose, cellobiose, and glucose were utilized by all species of the marine aerobic

cellulose-decomposing bacteria employed. More than 60% of the species employed utilized starch, sucrose, and galactose; about 50% of them utilized trehalose, lactose, maltose, mannose, fructose, arabinose, and xylose; 20-35% of them utilized inositol, mannitol, glycerin, raffinose, salicin, Ca-lactate, Ca-gluconate, and Na-alginate; less than 20% of them utilized erythritol, agar, inulin, and rhamnose. None of the species employed utilized dulcitol.

Among the species with which this work was concerned, *Vibrio marinoliquefaciens* had the greatest ability to utilize many kinds of organic compounds; *Cytophaga rosea*

Table 22. The growth response of the marine aerobic cellulose-decomposing bacteria to various nitrogen compounds.

Species \ Nitrogen compound	$NaNO_3$	$NaNO_2$	$(NH_4)_2SO_4$	NH_4Cl	Urea	Aspartic acid	Histidine	Peptone
Pseudomonas marinocellulosae	+	−	+	+	+	+	+	+
Vibrio aquamarinus	+	−	+	+	+	+	+	+
Vibrio purpureus	+	−	+	+	+	+	+	+
Vibrio purpureus var. *albus*	+	−	+	+	+	+	−	+
Vibrio gilvus	+	−	−	−	+	−	−	+
Vibrio albogilvus	+	−	+	+	+	+	+	+
Vibrio macerans	+	+	+	+	−	−	+	+
Vibrio marinoliquefaciens	+	−	+	+	+	+	+	+
Vibrio simplex	+	−	+	+	−	−	−	−
Vibrio ferrugineus	+	−	+	+	+	+	+	+
Vibrio brevis	+	−	+	+	+	+	+	+
Vibrio fulvus	+	−	+	+	+	+	+	+
Vibrio aurantiacus	+	−	+	+	+	+	+	+
Vibrio marinotypicus	+	−	+	+	+	+	+	+
Vibrio euryhalis	+	−	+	+	+	+	+	+
Vibrio marinoflavescens	+	−	+	+	+	+	+	+
Vibrio xanthus	+	−	+	+	+	+	+	+
Cytophaga haloflava	+	+	+	+	+	+	+	+
Cytophaga haloflava var. *nonreductans*	+	−	+	+	+	+	+	+
Cytophaga rosea	+	−	+	+	+	+	−	+

− = no growth, + = growth.

and *Vibrio ferrugineus* had the smallest.

In general, marine aerobic cellulose-decomposing bacteria belonging to genus *Vibrio* could utilize more kinds of organic compounds as a carbon source than those belonging to genus *Cytophaga*,—most of which could not utilize a variety of organic compounds but only a specialized few compounds such as cellulose, cellobiose, and glucose as the source of energy.

2. Utilization of nitrogen compounds by the marine aerobic cellulose-decomposing bacteria.

The growth response of the marine aerobic cellulose-decomposing bacteria to various nitrogen compounds which were respectively added, as a sole source of nitrogen, to the basal medium (K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; filtered sea water, 1000 ml, pH 7.2) containing a strip of filter paper was observed at 25°C.

The results obtained were given in Table 22.

These results indicate that the species employed did not vary widely in ability to utilize nitrogen compounds, and that most of them were evenly endowed with the ability to utilize a variety of nitrogen compounds.

Of all the compounds employed $NaNO_3$, $(NH_4)_2SO_4$, NH_4Cl, urea, aspartic acid, and peptone were utilized by the majority of the species employed, whereas histidine was utilized by about 80% of the species tested and $NaNO_2$ was utilized least frequently. $NaNO_2$ was not utilized by all the bacteria employed excepting *Vibrio macerans* and *Cytophaga haloflava*.

Of the bacteria employed, only one species *viz.*, *Vibrio simplex* could not utilize amino acids and peptone as the nitrogen source; this species appears to be not endowed with the ability to utilize organic nitrogen compounds at all.

VII. Mode of cellulose breakdown by the marine aerobic cellulose-decomposing bacteria.

The marine aerobic cellulose-decomposing bacteria easily attack filter paper. On this substrate these bacteria exerted a profound effect, causing a rapid maceration or a complete lysis of the fiber structure.

As shown previously, the external appearance of filter paper attacked by the marine aerobic cellulose-decomposing bacteria varied with the species employed. *Pseudomonas marinocellulosae*, *Vibrio aquamarinus*, *Vibrio purpureus*, *Vibrio purpureus* var. *albus*, *Vibrio gilvus*, *Vibrio albogilvus*, *Vibrio macerans*, and *Vibrio marinoliquefaciens* caused a rapid maceration of the fibre structure. In the cultures of these species, the paper contained was rapidly but incompletely destroyed and it became pulpy and macerated, so that when shaken with water it broke up at once into a large number of small fibre particles. While such a mode of destruction was a

characteristic of the *Vibrio* species predominating in summer and preferring a relatively high temperature for growth, it never occurred in cultures of *Cytophaga* species and *Vibrio* species predominating in winter.

The *Cytophaga* species (*Cytophaga haloflava*, *Cytophaga haloflava* var. *nonreductans*, and *Cytophaga rosea*) acted on filter paper in a manner similar to the *Vibrio* species predominating in winter (*Vibrio simplex*, *Vibrio ferrugineus*, *Vibrio brevis*, *Vibrio fulvus*, *Vibrio aurantiacus*, *Vibrio marinotypicus*, *Vibrio euryhalis*, *Vibrio marinoflavescens*, and *Vibrio xanthus*); the substrate was gradually, but completely destroyed, so that it became translucent at the air-water interface.

Imsenecki and Solntzeva[81] have used the expressive term "thawing" to describe the such lytic action on filter paper.

The growth on precipitated cellulose ("hydrocellulose") was also instructive. All the species employed could develop on mineral agar plates containing finely ground precipitated cellulose. As mentioned earlier, however, the development of these bacteria on the cellulose agar plates was not always accompanied by the formation of enzymatic fields of cellulose liquefaction around the growth. The enzymatic zones developing around the colonies varied in appearance widely with the species employed.

On the cellulose agar plates, colonies of *Vibrio simplex*, *Vibrio ferrugineus*, *Vibrio fulvus*, *Vibrio aurantiacus*, or *Vibrio euryhalis* which predominated in winter were surrounded by broad clear zones of complete cellulose dissolution (plate 21). The growth of *Cytophaga* species, however, was never accompanied by the formation of enzymatic fields of cellulose decomposition around the colonies; an attack on the cellulose took place only beneath the colonies (in the immediate neighborhood of the organisms*) (Plate 22).

And in the cases of *Vibrio purpureus*, *Vibrio purpureus* var. *albus*, *Vibrio aquamarinus*, *Vibrio gilvus*, and *Vibrio marinoliquefaciens* which predominated in summer, enzymatic zones of cellulose dissolution were found neither around the colonies, nor beneath them (Plate 23).

The above mentioned facts suggest that the mode of cellulose decomposition by the marine aerobic cellulose-decomposing bacteria varies with the species.

As for the mode of the bacterial decomposition of cellulose, it has been generally accepted for many years that cellulose decomposition is brought about by two enzyme systems, cellulase and cellobiase.

This pathway was first proposed by Pringsheim[82] as a result of his studies on cellulose decomposition by a crude culture of thermophilic organisms. Pringsheim was able to demonstrate that by arresting development of the bacteria in various

* This provides support for the contention of Winogradsky and others that the cytophagas must be in direct contact with cellulose in order to attack it.

ways cellobiose and glucose could be made to accumulate in the medium. Since that time, his conclusions have received confirmation through the works of KALNINS,[63] SIMOLA[64] and POCHON[65] using bacteria, and of VARTIOVAARA[66] with fungi. In general, these investigators have confirmed the accumulation of reducing substances (identified either as cellobiose or as glucose or as both) in cellulose-decomposing cultures, and have established[64] the cellulolytic activity of cell-free extracts.

WINOGRADSKY[67] ignored the PRINGSHEIM's formulation of a simple hydrolytic route and developed an elaborate theory of oxidative decomposition which he considered to hold for cellulose breakdown by all aerobic bacteria. WINOGRADSKY observed that filter paper attacked by aerobic bacteria reacted in a manner which indicated that it was similar in certain respects to the rather ill-defined material known as oxycellulose, which results from a partial chemical oxidation of cellulose. Attacked portions of filter paper were claimed to have acidic properties, as shown particularly by their intense staining with methylene blue, a basic dye.

The conclusion which WINOGRADSKY drew from these observations was that the cellulose underwent a direct attack through the oxidation of the protruding carbinol groups to aldehydic and ultimately to carboxylic groups, with the resultant formation and accumulation of "oxycellulose".

WINOGRADSKY's theory has been accepted by LOICJANSKAJA[68] and by WALKER and WARREN.[69] The latter authors, using *Cytophaga hutchinsonii*, isolated the mucilaginous material and studied it chemically. They were able to show that it was a polyuronide, and concluded that it was an "acidic type of oxycellulose" formed from the partial breakdown of cellulose.

IMSENECKI and SOLNTZEVA[61] were the first to perceive some of the flaws in WINOGRADSKY's theory. They discovered that attacked filter paper was stained just as heavily by acidic as by basic dyes, and from this concluded very reasonably that it was the accumulated bacterial cells, rather than any "oxycellulose", which actually absorbed and retained methylene blue.

NORMAN and his associates[70][71] have also criticized the oxydative theory. They stated that such an oxidative attack presupposed the existence of an oxidative exoenzyme, which was highly improbable. Furthermore, no energy could be obtained by the cells until water-soluble fragments had been produced, so that there was no good apparent reason why oxycellulose should accumulate.

Thus the factual basis of the oxidative theory has become highly doubtful.

After a comprehensive study on aerobic cellulose-decomposing bacteria STANIER[49] suggested that the fission of cellulose molecule by the bacteria might take place either by the continued lopping off from the end of the chain of short terminal sections containing from one to a few glucose units, or by an approximately central splitting of the chain repeated on the resulting fragments with the production of

shorter and shorter lengths. In the former case, a small amount of enzyme should give to detectable amounts of reducing sugars. In the latter case, however, even a large quantity of the enzyme would yield only very small amounts of reducing sugars when acting on a highly polymerized cellulose.

Recently, REESE and co-workers[72-74] concluded from the results of their experiments concerning the biological degradation of soluble cellulose derivatives that the classical enzyme "cellulase" (PRINGSHEIM)[68], presumably converting native cellulose to sugars, consisted of at least two systems. The first step, designated as C_1, occured preliminary to hydrolysis of the straight chain by Cx. The sequence was diagramed as follows:

$$\text{Native cellulose} \xrightarrow{C_1} \underbrace{\text{shorter linear polyanhydroglucose chains}} \xrightarrow{Cx} \underbrace{\text{soluble, small molecules, capable of diffusion into the cell}}$$

Non cellulolytic organisms

Cellulolytic organisms

TERUI, FUJIWARA, and KAWAI[75][76] reported the occurrence of the cellulase of "weathering" type in cultures of an aerobic cellulose-decomposing bacteria (*Bacillus hydrolyticus*). The residual cellulose attacked by this organism became fragile as compared with sterile controls. For the quantitative expression of the enzymatic action of such mode, TERUI and his co-workers adopted properly an index (R) defined as follows:

$$R = 100 \cdot (K - K_0) / K_0$$

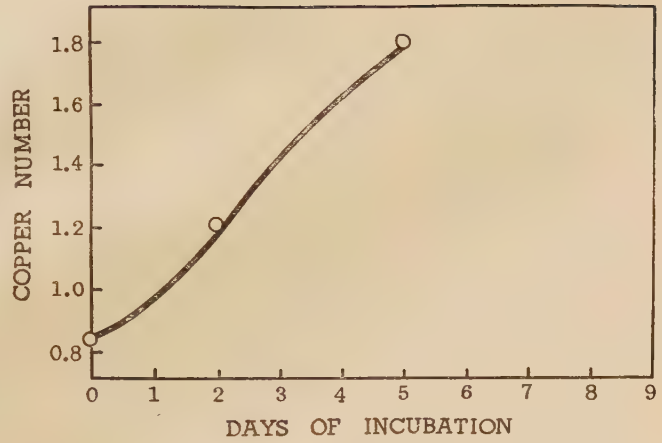
where K is a velocity constant of hydrolysis of residual cellulose with $N\text{-}H_2SO_4$ at 180°C, and K_0 is that of intact one in a control. In the case of *Bacillus hydrolyticus*, R ranged from 43 to 93 in 15 day old cultures at 30°C, depending on the composition of the media. In contrast to the cellulase of "weathering" type, the already known cellulase was called as the cellulase of "liquefying" or "saccharogen" type by these workers.

In order to provide experimental evidence to the aforementioned visual observations on the mode of cellulose decomposition by the marine aerobic cellulose-decomposing bacteria, changes in chemical natures such as copper number and index (R) of the cellulose were studied in this chapter.

The experimental method employed for observing the changes in copper number of cellulose attacked by the bacteria was as follows:

The marine aerobic cellulose-decomposing bacteria were grown with continuous aeration at 25°C in a synthetic medium containing cellulose ($NaNO_3$, 0.5 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; finely ground cellulose 20.0 g; and filtered sea water, 1000 ml, pH 7.2).

After the desired period of incubation, the residual cellulose was recovered by passing the cultures through a glass filter and then washing repeatedly with a large



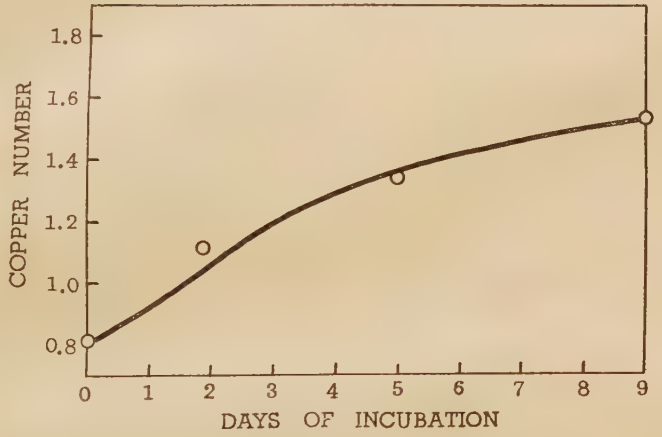
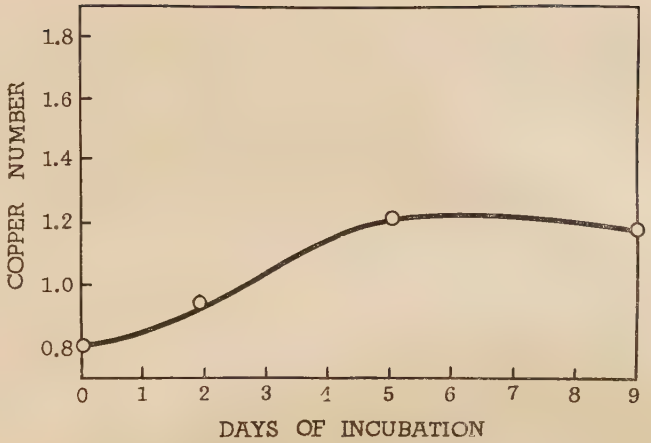


Fig. 4-b. Changes in the copper number of residual cellulose during attack by *Vibrio purpureus* (upper), and *Vibrio purpureus* var. *albus* (lower).



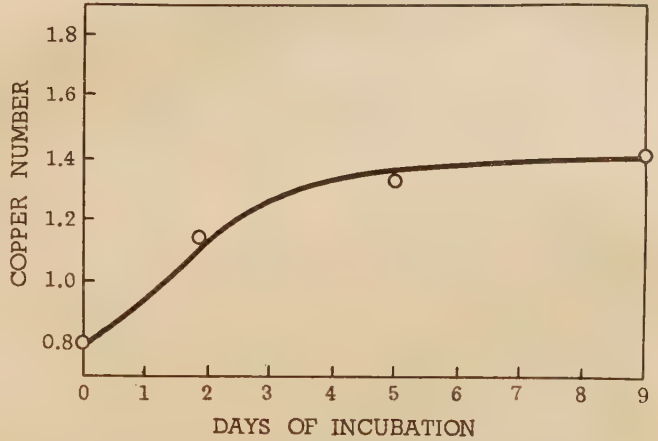
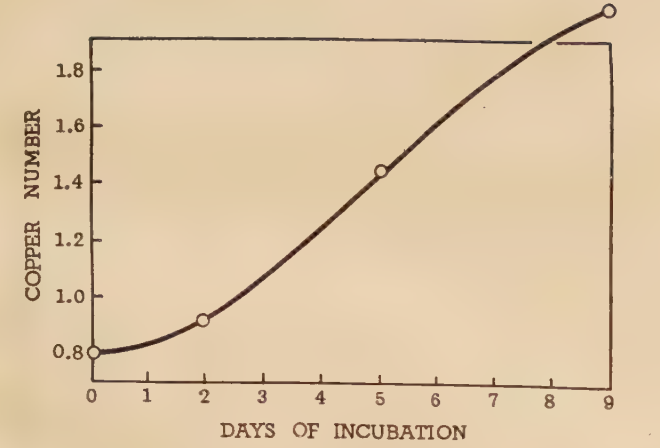


Fig. 4-a. Changes in the copper number of residual cellulose during attack by *Pseudomonas marinocellulosae* (upper), and *Vibrio aquamarinus* (lower).



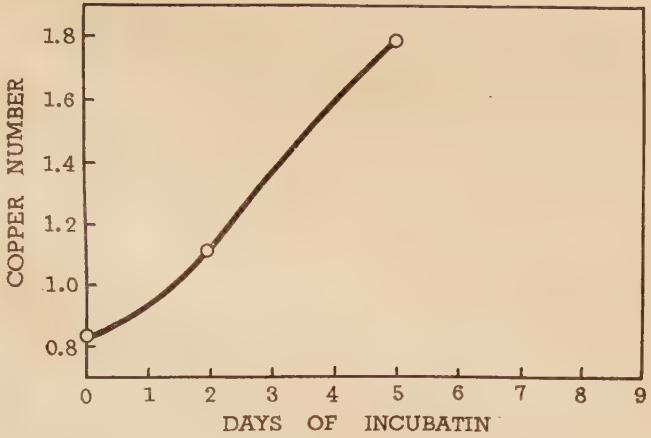
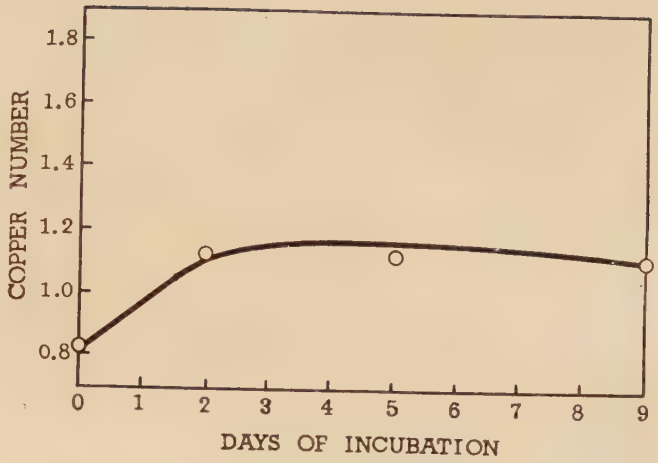


Fig. 4-d. Changes in the copper number of residual cellulose during attack by *Vibrio macerans* (upper), and *Vibrio marinoliquefaciens* (lower).



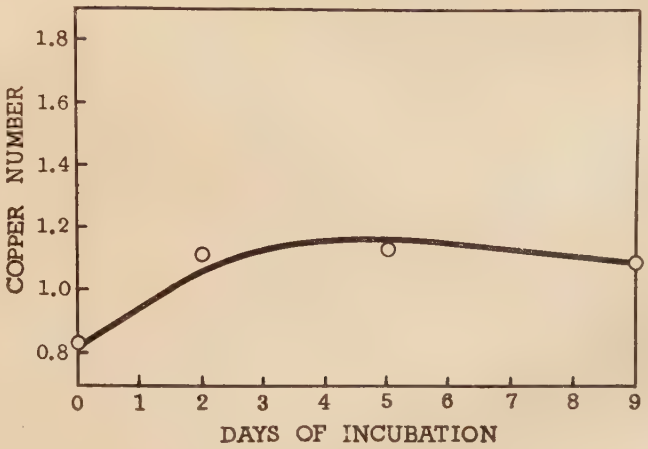
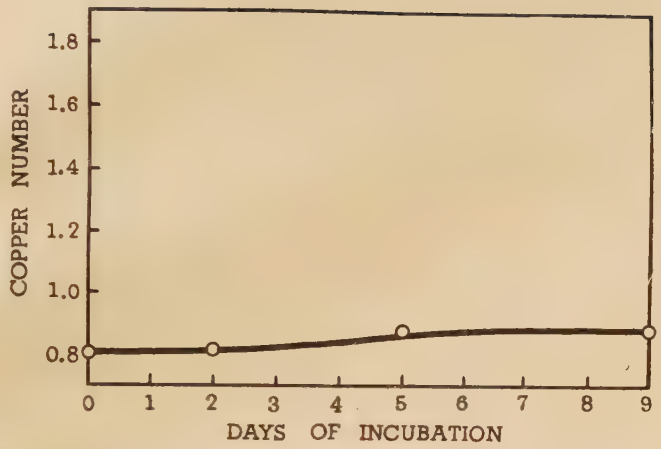


Fig. 4-c. Changes in the copper number of residual cellulose during attack by *Vibrio gilvus* (upper), and *Vibrio albogilvus* (lower).



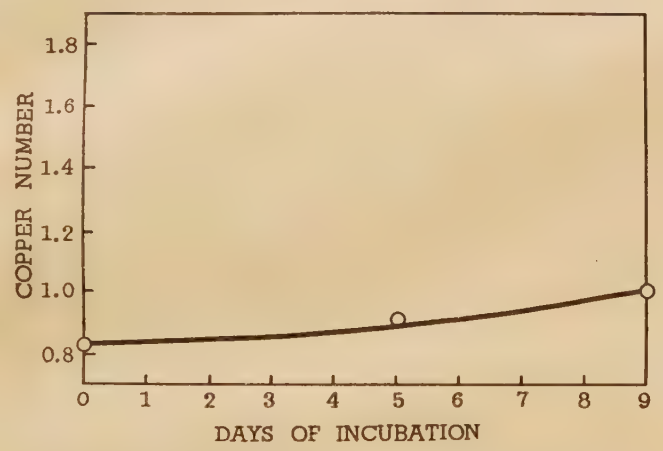
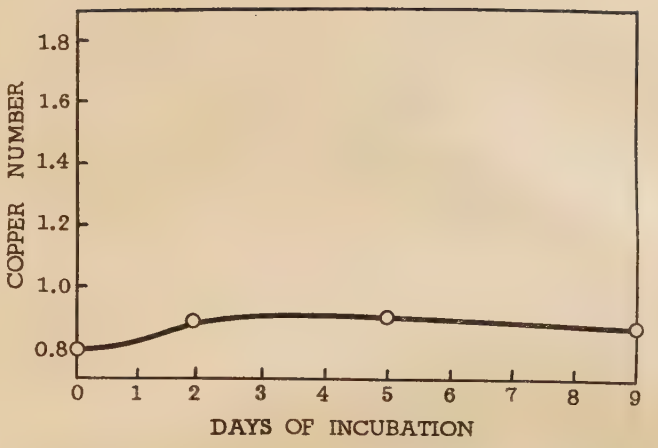


Fig. 4-f. Changes in the copper number of residual cellulose during attack by *Vibrio brevis* (upper), and *Vibrio fulvus* (lower).



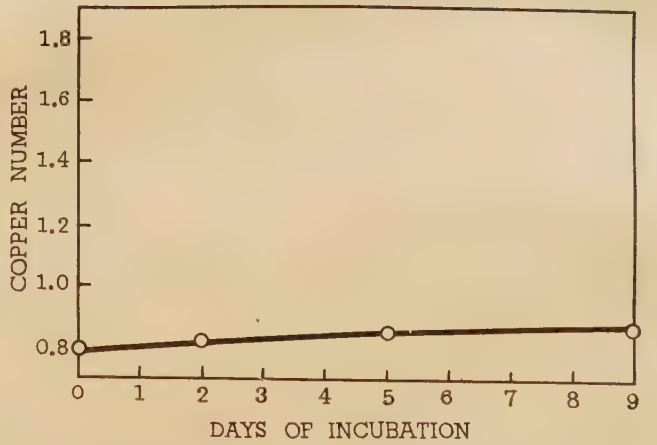
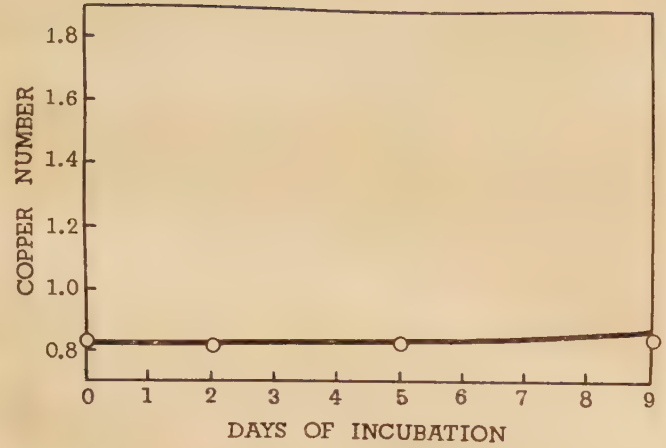


Fig. 4-e. Changes in the copper number of residual cellulose during attack by *Vibrio simplex* (upper), and *Vibrio ferrugineus* (lower).



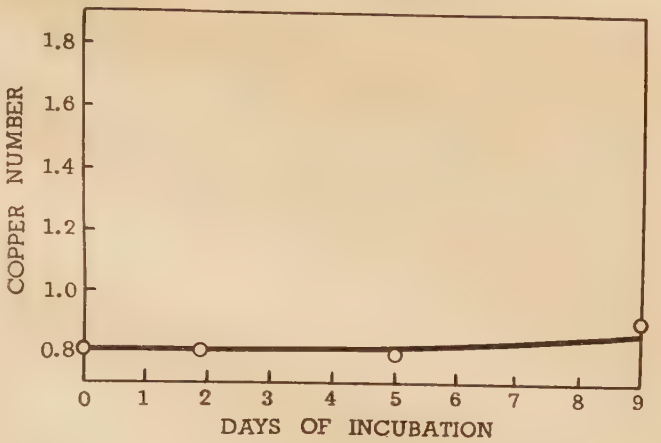
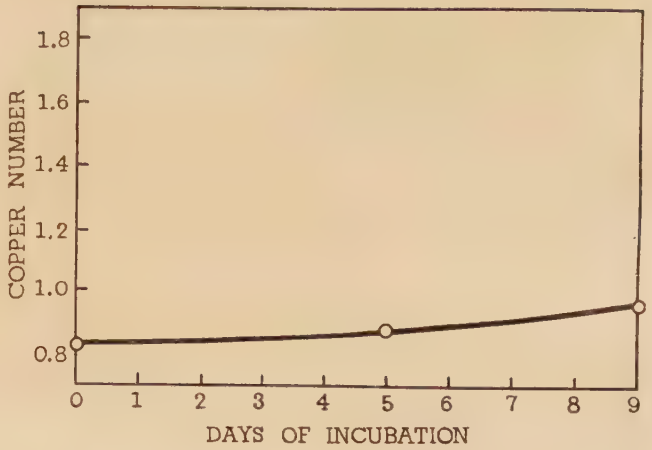


Fig. 4–h. Changes in the copper number of residual cellulose during attack by *Vibrio euryhalis* (upper), and *Vibrio marinoflavescens* (lower).



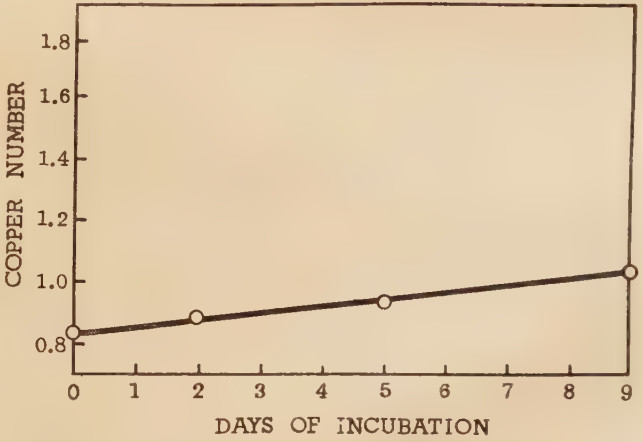
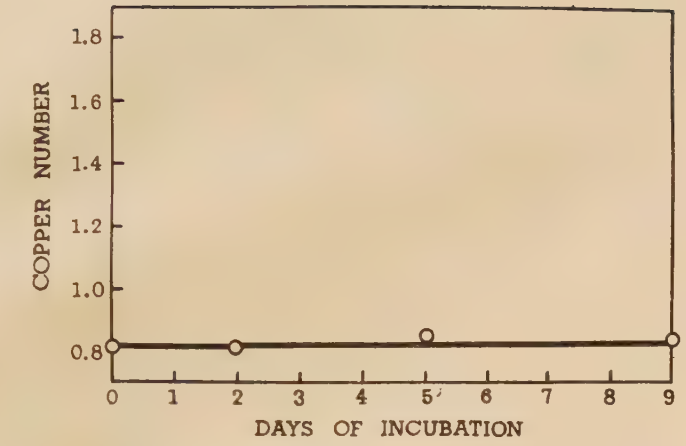


Fig. 4–g. Changes in the copper number of residual cellulose during attack by *Vibrio aurantiacus* (upper), and *Vibrio marinotypicus* (lower).



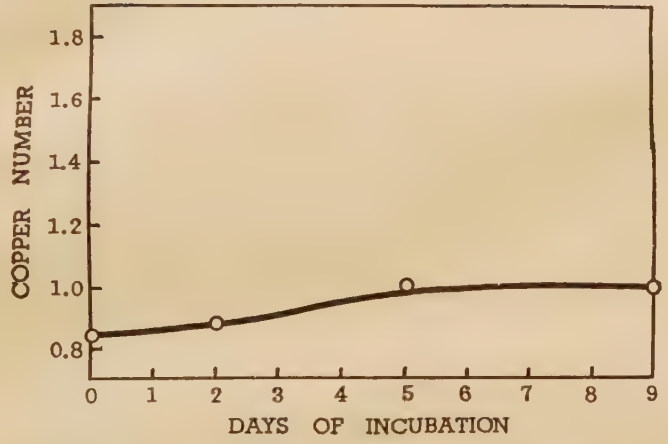
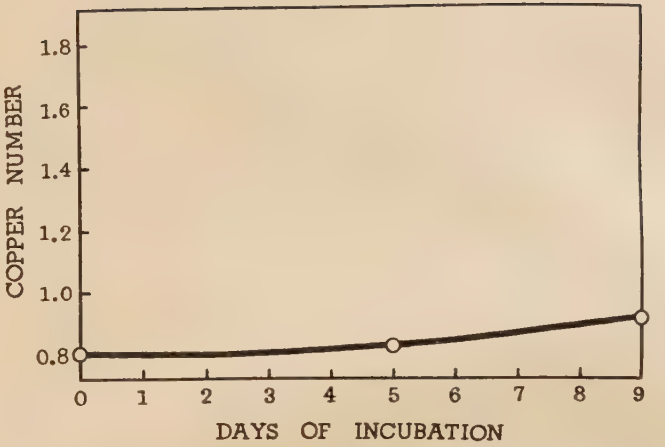


Fig. 4–j. Changes in the copper number of residual cellulose during attack by *Cytophaga haloflava* var. *nonreductans* (upper), and *Cytophaga rosea* (lower).



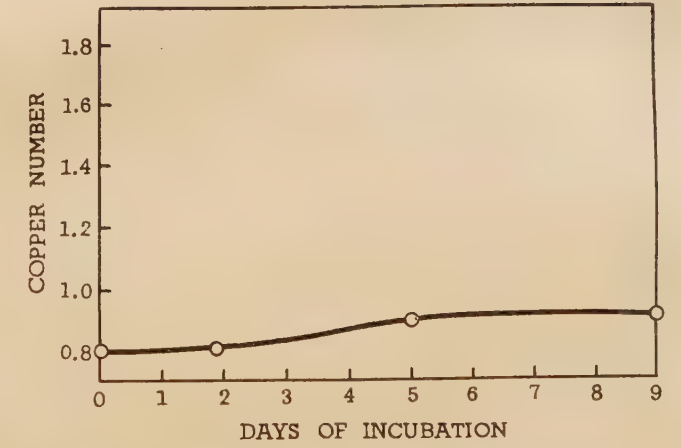


Fig. 4–i. Changes in the copper number of residual cellulose during attack by *Vibrio xanthus* (upper), and *Cytophaga haloflava* (lower).

amount of hot water until the Cl^- in the residual cellulose was completely removed.

The copper number of the above residual cellulose was determined by HEYES' method[77].

The results obtained were as shown in Fig. 4 (a–j).

These results indicate that the organisms employed fell into two groups with respect to the mode of cellulose decomposition.

Of these species, *Vibrio simplex*, *Vibrio ferrugineus*, *Vibrio brevis*, *Vibrio fulvus*, *Vibrio marinoflavescens*, *Vibrio aurantiacus*, *Vibrio marinotypicus*, *Vibrio euryhalis*, *Vibrio xanthus*, *Cytophaga haloflava*, *Cytophaga haloflava* var. *nonreductans*, and *Cytophaga rosea* liquefied cellulose, but hardly changed the nature of the residual cellulose. In the cases of these cultures the fission of long chain molecules of cellulose might be accomplished by the continued lopping off from the end of the chain of short terminal sections containing from one to a few glucose units.

On the other hand, *Vibrio purpureus*, *Vibrio purpureus* var. *albus*, *Vibrio aquamarinus*, *Vibrio gilvus*, *Vibrio albogilvus*, *Vibrio macerans*, *Vibrio marinoliquefaciens*, and *Pseudomonas marinocellulosae* markedly denaturalized cellulose during their attack on it. The copper number of the residual cellulose attacked by these species remarkably increased with the lapse of time of incubation. In these cases the fission of cellulose molecules might be accomplished by the approximately central splitting of the chain repeated on the resulting fragments with the production of shorter and shorter lengths.

The changes in index (R)[83)84)] of cellulose which was attacked by several representatives of aforementioned two groups of marine aerobic cellulose-decomposing bacteria were observed by the following method:

The bacteria were grown at 25°C in 200 ml of a synthetic medium containing cellulose ($NaNO_3$, 0.5 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; finely ground cellulose, 20.0 g; and filtered sea water, 1000 ml, pH 7.2). As the inoculants cultures of *Vibrio marinoflavescens*, *Vibrio purpureus*, and *Vibrio purpureus* var. *albus* were employed. After 5 days' incubation, each culture was passed through a glass filter and the residual cellulose was recovered. This residual cellulose was throughly washed by a large amount of hot water until the Cl^- in the cellulose was completely removed.

Index (R) of the residual cellulose was determined by the method reported by TERUI and his co-workers.[75)76)]

The results obtained were presented in Table 23.

Table 23. Index (R) of residual cellulose after deterioration by marine aerobic cellulose-decomposing bacteria for 5 days at 25°C. Index (R) was defined as follows:

$$R = \frac{K - K_0}{K_0} \times 100$$

where K is a velocity constant of hydrolysis of residiual cellulose with 2N-H_2SO_4 at 100°C, and K_0 is that of intact one in a control.

Organism inoculated	Index (R)
Vibrio marinoflavescens	6.8
Vibrio purpureus	33.4
Vibrio purpureus var. *albus*	20.1

As shown above, R ranged from 6.8 to 33.4 in 5 days old cultures at 25°C, depending on the species employed.

This result indicates that the residual cellulose attacked by *Vibrio purpureus* and *Vibrio purpureus* var. *albus* were more fragile than that attacked by *Vibrio marinoflavescens*.

With the aim of elucidating the cellulose-liquefying activity of marine aerobic cellulose-decomposing bacteria the changes in weight of cellulose attacked by the aforementioned three species were observed respectively by the following method:

The bacteria were grown at 25°C in 50 ml of a synthetic medium containing cellulose ($NaNO_3$, 0.5 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; finely ground cellulose*, 20.0 g; and filtered sea water, 1000 ml, pH 7.2).

After the desired periods of incubation the content of each flask was passed through glass filter which had been preliminarily weighed after 2 hours' treatment at 105°C. The residual cellulose thus recovered was washed successively by 200 ml of 1.25% H_2SO_4, 200 ml of 1.25% NaOH, a large amount of distilled water, 10 ml of ethanol, and 10 ml of ethylether. After being thoroughly washed, the residual cellulose was dried at 105°C until its weight became constant and thereafter it was weighed together with the glass filter.

The weight of residual cellulose calculated from this value was as shown in Table 24.

Table 24. Weight of residual cellulose after deterioration by marine aerobic cellulose-decomposing bacteria for 0, 2, 5, 7, 10, and 20 days. Results were shown in per cent.

Organism inoculated	Incubation time in days					
	0	2	5	7	10	20
Vibrio marinoflavescens	100	98.3	96.2	95.3	93.3	87.3
Vibrio purpureus	100	98.7	98.4	96.3	95.4	90.7
Vibrio purpureus var. *albus*	100	99.2	98.7	98.5	97.8	96.5

* Before it was added to each culturing flask, cellulose was preliminarily dried at 105°C until its weight became constant and then weighed correctly.

This result indicates that *Vibrio marinoflavescens* was superior to *Vibrio purpureus* and *Vibrio purpureus* var. *albus* with respect to the cellulose liquefying activity.

From Fig. 4, and Tables 23 and 24 it may be concluded that the cellulose decomposition by *Vibrio marinoflavescens* was of the "liquefying" type and that by *Vibrio purpureus* and *Vibrio purpureus* var. *albus* was of the "weathering" type.

In general, it seems likely that the mode of cellulose decomposition by the majority of *Vibrio* species predominating in summer was of the "weathering" type and those by *Cytophaga* species and the majority of *Vibrio* species preferring relatively low temperatures for growth were of the "liquefying" type. The velocity of the cellulose deterioration of the "weathering" type generally seemed to be higher than that of the "liquefying" type.

It seems, therefore, that the rapid deterioration of cotton fishing nets submerged in sea water during summer months may mainly be caused by the rapid action of the microorganisms causing the cellulose decomposition of the "weathering" type.

VIII. Breakdown of cellulose derivatives by the marine aerobic cellulose-decomposing bacteria.

The relationship between the molecular structure of cellulose and its susceptibility to microorganisms appears to be one of considerable specificity.

In this chapter the author studied the behavior of the marine aerobic cellulose-decomposing bacteria on several samples of cellulose acetate which were substituted in various degrees.

The experimental method employed was as follows:

Samples of cellulose acetate with various degrees of substitution were prepared from the acetylation of purified yarns of ramie (60 counts: single yarn). The acetyl content of each sample was 0, 7.7, 26.6, 31.2, 37.0, 46.5, 53.7, and 61.5%, respectively.

The basal medium employed in this experiment contained $NaNO_3$, 0.05%; K_2HPO_4, 0.1%; $MgSO_4 \cdot 7H_2O$, 0.05%; and $FeSO_4 \cdot 7H_2O$, 0.001%. These salts were dissolved in filtered sea water, and the pH was adjusted to 7.2. Suitable aliquots of this medium were dispensed into test tubes containing several pieces of the desired sample of the above mentioned cellulose acetate so as to immerse the acetate sample partly in it, and the whole was sterilized by autoclaving. The media thus prepared were inoculated with marine aerobic cellulose-decomposing bacteria and incubated at 25°C.

The growth of the bacteria was observed visually after 10 days' incubation.

The results obtained were as shown in Table 25.

These results indicate that cellulose acetate with acetyl contents varying from 26.6 to 61.5 per cent were all totally resistant to attack of the marine aerobic cellulose-decomposing bacteria.

Table 25. Growth of the marine aerobic cellulose-decomposing bacteria on cellulose acetate of different acetyl content.

Organism \ Acetyl content in per cent	0	0*	7.7*	26.6*	31.2*	37.0*	46.5*	53.7*	61.5*
Pseudomonas marinocellulosae	|||	|||	++	−	−	−	−	−	−
Vibrio aquamarinus	++	|||	−	−	−	−	−	−	−
Vibrio purpureus	|||	|||	−	−	−	−	−	−
Vibrio purpureus var. *albus*	++	|||	−	−	−	−	−	−	−
Vibrio gilvus	|||	|||	−	−	−	−	−	−
Vibrio albogilvus	|||	|||	−	−	−	−	−	−
Vibrio macerans	++	|||	−	−	−	−	−	−	−
Vibrio marinoliquefaciens	++	|||	−	−	−	−	−	−	−
Vibrio simplex	|||	|||	++	−	−	−	−	−
Vibrio ferrugineus	++	|||	±	−	−	−	−	−	−
Vibrio brevis	|||	|||	++	−	−	−	−	−
Vibrio fulvus	++	|||	++	±	−	−	−	−	−
Vibrio aurantiacus	|||	|||	±	−	−	−	−	−
Vibrio marinotypicus	|||	|||	++	±	−	−	−	−
Vibrio euryhalis	|||	|||	++	±	−	−	−	−
Vibrio marinoflavescens	|||	|||	++	−	−	−	−	−
Vibrio xanthus	|||	|||	++	−	−	−	−	−
Cytophaga haloflava	|||	|||	++	−	−	−	−	−
Cytophaga haloflava var. *nonreductans*	|||	|||	+	−	−	−	−	−
Cytophaga rosea	|||	|||	+	−	−	−	−	−

− = no growth, ||| = maximum growth.

* Fat contained in these samples was preliminarily removed by the extraction with methanol before acetylation.

On the samples of cellulose acetate with an acetyl content of 7.7 per cent, however, about a half of the organisms tested grew as well as on the unacetylated cords of ramie; none of the cellulose-decomposing vibrios predominating in summer could attack such samples of cellulose acetate, but the majority of the other organisms tested were able to utilize these samples.

In the samples with acetyl contents varying from 26.6 to 61.5 per cent there were more than one substituent on every anhydroglucose unit, but in the samples

with acetyl content of 7.7 per cent there was one or no substituent on every anhydroglucose unit. Therefore, it may be suggested from these results that cellulose acetate, with at least one acetyl group on every anhydroglucose unit, is completely resistant to attack of the marine aerobic cellulose-decomposing bacteria.

This presumption is supported by the works of SIU[73,75], who found that cyanoethylated celluloses with more than one substituent per anhydroglucose unit were totally resistant to the fungus within the experimental limits of error and suggested that as long as there is at least one substituent on every anhydroglucose unit in cellulose molecules, complete resistance to microorganisms is obtained. Table 26 presents data on the susceptibility of cyanoethylated cellulose obtained by SIU.[73] He stated that partial utilization of preparation No. 2 which had 1.02 substituents per anhydroglucose unit was probably due to a nonhomogeneity of cyanoethylation of the cellulose.

Another significant piece of supporting data on the microbiological resistance of monosubstituted anhydroglucose units in a cellulose chain was given by REESE, SIU, and LEVINSON[73,75] (Fig. 5). Plotted in the graph is the rate of hydrolysis of

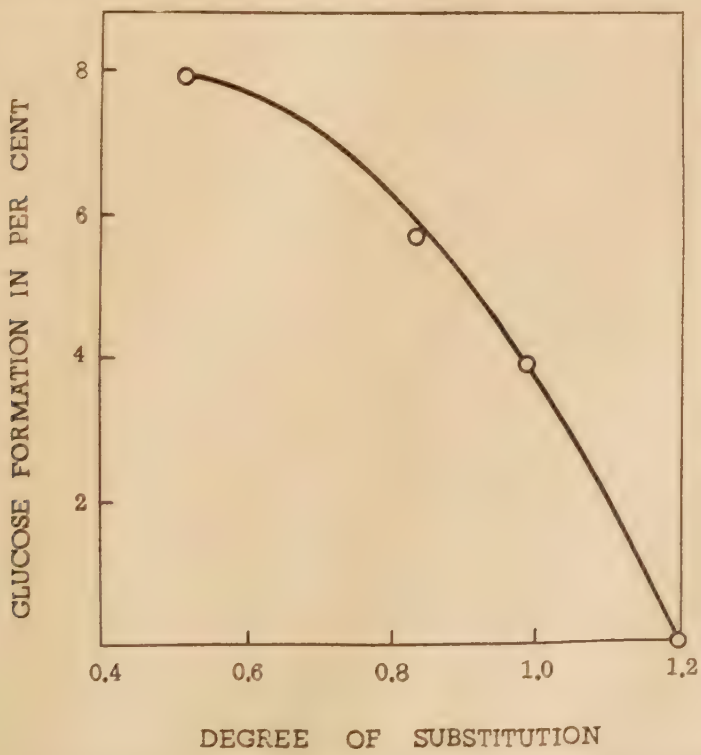


Fig. 5. Action of metabolic filtrate from *Aspergillus fumigatus* on partially substituted carboxymethylcelluloses. (From REESE, SIU, and LEVINSON[73])

Table 26. Utilization of Cyanoethylcellulose by *Myrothecium Verrucaria*. (From SIU[78])

Sample No.	% N	No. of substituents per anhydroglucose unit	% Weight loss in 13 days
1	0	0	80
2	6.64	1.02	29
3	8.43	1.43	6
4	9.98	1.85	2
5	10.50	2.02	4
6	11.44	2.35	0
7	12.87	2.9	0

partially substituted carboxyme-thylcellulose against the degree of substitution. The rapid decline at 1 degree of substitution (1 D. S.) and the complete resistance at 1.2 D. S. are considered to support the above presumption.

As was suggested by SIU[85] it appears as if the nature of the substituent itself is of only secondary importance, if any, in determining the resistance of the cellulose derivative to microorganisms.

The data given by SIU, DARBY, BURKHOLDER, and BARGHOORN[79] (Table 27) seem to uphold this statement.

GREATHOUSE[80] has also observed that as long as the degree of substitution was over one, regardless of the identity of the substituent, no digestion of the cellulose derivative by the microorganisms took place.

The resistance of cellulose triacetate to attack by soil microorganisms has been reported by several investigators.[79][81] They found that cellulose triacetate with degrees of polymerization varying from 61 to 395 were all totally resistant to microbiological attack. The samples were tested against *Gliomastix convoluta*, *Curvularia lunata*, *Sporocytophaga myxococcoides*, *Myrothecium verrucaria*, and *Aspergillus flavipes* with the same result.

From the above mentioned facts, it is thought that fishing nets made of cellulose acetate with acetyl contents of more than 26.6 per cent will hardly rot either during immersion in the sea or during storage after fishing.

IX. Disinfecting effect of some chemical agents upon the marine aerobic cellulose-decomposing bacteria.

The second general approach toward minimizing the action of microorganisms on cellulose textiles of fishing nets is thought to be the application of compounds which act as poisons for marine cellulose-decomposing bacteria. These compounds either inhibit the microorganism from further development (bacteriostatic) or bring about death (bactericidal).

In this chapter several organic and inorganic disinfectants were studied with respect to their cidal effects on the marine aerobic cellulose-decomposing bacteria.

Experimental method employed was as follows:

As the disinfectants $CuSO_4$, $HgCl_2$, phenol, pentachlorphenol, 5-nitro-2-furfuryl

Table 27. Growth of *Myrothecium verrucaria* on cellulose derivatives. (From Siu, Darby, Burkholder, and Barghoon[79])

Preparation	Description	% Weight loss
Filter paper	Ground to 60 mesh	69
Oxidized cellulose	Prepared with nitrogen oxide	11
Methylcellulose	D. S. > 1.0	8
Methylcellulose	D. S. = 2.0	8
Ethylcellulose	D. S. = 2.25 to 2.58	0
Cellulose acetate	D. S. = 1.0; 22.3 % AcO	0
Cellulose triacetate		0
Cellulose acetate butyrate	16 % butyryl	0
Cellulose acetate hydrogen phthalate		8
Cellulose acetate stearate		1
Tosylcellulose	D. S. = 1.18 to 2.01	0
Iodotosylcellulose	D. S. = {0.60 to 0.86 iodotosyl 0.58 to 1.15 tosyl	2
Carboxymethylcellulose	D. S. = 0.067	56
Carboxymethylcellulose, sodium salt	D. S. = 0.2	66
Carboxymethylcellulose, alminium salt	D. S. = 0.067	56

semicarbazone, and cutch were employed. These compounds were respectively added in various concentrations to the cultures of the marine aerobic cellulose-decomposing bacteria which had grown for 7 days at 25°C in a sea water medium (cellobiose, 1.0 g; $NaNO_3$, 0.5 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; and filtered sea water, 1000 ml, pH 7.2).

After desired periods of the contact with the disinfectant at 15°C a small amount (3 loopful) of the culture was transferred to 10 ml of the recovery culture medium having the same composition as above mentioned preculture medium, where the presence or absence of growth was observed by 7 days' incubation at 25°C.

The results obtained were as shown in Tables 28, 29, 30, 31, 32, and 33.

These tables show that among the compounds tested $HgCl_2$ had the maximum potency in respect to the disinfecting effect upon the marine aerobic cellulose-decomposing bacteria, *viz.*, all of these bacteria were perfectly disinfected at as low a concentration as 1 : 10,000, and the majority of them in a concentration of 1 : 100,000.

Mercuric chloride has already been tried on fishnet twine and found to be more

Table 28. Disinfection of the marine aerobic cellulose-decomposing bacteria with various concentrations of $CuSO_4$ at 15°C.

Concentration of $CuSO_4$ / Contact time in minutes / Organism	1 : 100		1 : 1,000		1 : 10,000		1 : 100,000		0
	10	30	10	30	10	30	10	30	
Pseudomonas marinocellulosae	−	−	−	−	|||	|||	|||	|||	|||
Vibrio aquamarinus	−	−	−	−	|||	|||	|||	|||	|||
Vibrio purpureus	−	−	−	−	−	−	|||	|||	|||
Vibrio purpureus var. *albus*	−	−	−	−	|||	|||	|||	|||	|||
Vibrio gilvus	−	−	−	−	−	−	|||	|||	|||
Vibrio albogilvus	−	−	−	−	|||	|||	|||	|||	|||
Vibrio macerans	−	−	−	−	−	−	|||	|||	|||
Vibrio marinoliquefaciens	−	−	−	−	|||	|||	|||	|||	|||
Vibrio simplex	−	−	−	−	−	−	|||	−	|||
Vibrio ferrugineus	−	−	−	−	−	−	|||	−	|||
Vibrio brevis	−	−	−	−	|||	|||	|||	|||	|||
Vibrio fulvus	−	−	−	−	|||	|||	|||	|||	|||
Vibrio aurantiacus	−	−	−	−	−	−	|||	−	|||
Vibrio marinotypicus	−	−	|||	−	|||	|||	|||	|||	|||
Vibrio euryhalis	−	−	−	−	|||	|||	|||	|||	|||
Vibrio marinoflavescens	−	−	−	−	|||	|||	|||	|||	|||
Vibrio xanthus	−	−	−	−	−	−	|||	−	|||
Cytophaga haloflava	−	−	−	−	−	−	|||	|||	|||
Cytophaga haloflava var. *nonreductans*	−	−	−	−	−	−	±		−	|||
Cytophaga rosea	−	−	−	−	−	−	|||	−	|||

− = no growth; ||| = maximum growth.

effective than copper sulfate.[82)83)] $CuSO_4$, phenol, penta chlor phenol, and 5-nitro-2-furfuryl semicarbazone were more or less toxic to the marine aerobic cellulose-decomposing bacteria in concentrations lower than one per cent. However, cutch, which has been widely used as the preservative of fishing nets since olden times, was almost not toxic to these bacteria at as high a concentration as one per cent.

Although the fact that a compound was toxic to marine cellulose-decomposing effective than copper sulfate.[82)83)]

Table 29. Disinfection of the marine aerobic cellulose-decomposing bacteria with various concentrations of $HgCl_2$ at 15°C.

Concentration of $HgCl_2$	1 : 10,000		1 : 100,000		1 : 1,000,000		0
Contact time in minutes / Organism	10	30	10	30	10	30	
Pseudomonas marinocellulosae	—	—	—	—	||||| ||||| |||||
Vibrio aquamarinus	—	—	—	—	||||| ||||| |||||
Vibrio purpureus	—	—	—	—	||||| ||||| |||||
Vibrio purpureus var. *albus*	—	—	—	—	||||| ||||| |||||
Vibrio gilvus	—	—	—	—	||||| ||||| |||||
Vibrio albogilvus	—	—	—	—	||||| ||||| |||||
Vibrio macerans	—	—	—	—	||||| ||||| |||||
Vibrio marinoliquefaciens	—	—	||||| ||||| ||||| ||||| |||||
Vibrio simplex	—	—	—	—	||||| ||||| |||||
Vibrio ferrugineus	—	—	—	—	||||| ||||| |||||
Vibrio brevis	—	—	—	—	||||| ||||| |||||
Vibrio fulvus	—	—	—	—	||||| ||||| |||||
Vibrio aurantiacus	—	—	—	—	||||| ||||| |||||
Vibrio marinotypicus	—	—	||||| —	||||| ||||| |||||
Vibrio euryhalis	—	—	—	—	||||| ||||| |||||
Vibrio marinoflavescens	—	—	—	—	||||| ||||| |||||
Vibrio xanthus	—	—	—	—	||||| ||||| |||||
Cytophaga haloflava	—	—	—	—	||||| ||||| |||||
Cytophaga haloflava var. *nonreductans*	—	—	—	—	||||| ||||| |||||
Cytophaga rosea	—	—	—	—	||||| ||||| |||||

— = no growth; ||||| = maximum growth.

bacteria when tested by the above mentioned method, is no guarantee of its effectiveness as a preservative for cotton fishing nets immersed in the sea, this fact shows that such a compound is effective as a preservative for cotton nets during storage after fishing.

As previously stated, a large number of marine aerobic cellulose-decomposing bacteria which had attached to and developed on cotton fishing nets during immer-

Table 30. Disinfection of the marine aerobic cellulose-decomposing bacteria with various concentrations of phenol at 15°C

Concentration of phenol / Contact time in minutes / Organism	1 : 100		1 : 1,000		1 : 2,000		0
	10	30	10	30	10	30	
Pseudomonas marinocellulosae	−	−	++++	++++	++++	++++	++++
Vibrio aquamarinus	−	−	++++	++++	++++	++++	++++
Vibrio purpureus	−	−	±	±	++++	++++	++++
Vibrio purpureus var. *albus*	−	−	++++	++++	++++	++++	++++
Vibrio gilvus	−	−	±	−	++++	++++	++++
Vibrio albogilvus	−	−	++++	++++	++++	++++	++++
Vibrio macerans	−	−	++++	++++	++++	++++	++++
Vibrio marinoliquefaciens	−	−	++++	++++	++++	++++	++++
Vibrio simplex	−	−	++++	−	++++	++++	++++
Vibrio ferrugineus	−	−	−	−	++++	±	++++
Vibrio brevis	−	−	++++	±	++++	++++	++++
Vibrio fulvus	−	−	++++	±	++++	++++	++++
Vibrio aurantiacus	−	−	++++	±	++++	++++	++++
Vibrio marinotypicus	−	−	++++	++++	++++	++++	++++
Vibrio euryhalis	−	−	++++	++++	++++	++++	++++
Vibrio marinoflavescens	−	−	++++	++++	++++	++++	++++
Vibrio xanthus	−	−	++++	±	++++	++++	++++
Cytophaga haloflava	−	−	++++	++++	++++	++++	++++
Cytophaga haloflava var. *nonreductans*	−	−	++++	−	++++	++++	++++
Cytophaga rosea	−	−	±	−	++++	++++	++++

− = no growth; ++++ = maximum growth.

sion in sea water, survived for a considerably long period during storage on land under wet as well as dry conditions after fishing. It appears that these bacteria attack the nets to a certain extent during the period of storage, and that not a small percentage of them may survive until the nets are used again. The nets bearing the cellulose-decomposing bacteria which have survived in such a way are probably rotted more rapidly than the nets which are newly immersed in sea water.

Table 31. Disinfection of the marine aerobic cellulose-decomposing bacteria with various concentrations of penta chlor phenol at 15°C.

Concentration of PCP. / Contact time in minutes / Organism	1 : 2,000		1 : 10,000		1 : 100,000		1 : 1,000,000		0
	10	30	10	30	10	30	10	30	
Pseudomonas marinocellulosae	−	−	|||	−	|||	|||	|||	|||
Vibrio aquamarinus	−	−	−	−	|||	|||	|||	|||	|||
Vibrio purpureus	−	−	−	−	|||	−	|||	|||	|||
Vibrio purpureus var. *albus*	−	−	−	−	|||	|||	|||	|||	|||
Vibrio gilvus	−	−	−	−	|||	|||	|||	|||	|||
Vibrio albogilvus	−	−	−	−	|||	|||	|||	|||	|||
Vibrio macerans	−	−	−	−	|||	|||	|||	|||	|||
Vibrio marinoliquefaciens	|||	|||	|||	|||	|||	|||	|||	|||
Vibrio simplex	−	−	−	−	|||	|||	|||	|||	|||
Vibrio ferrugineus	−	−	|||	||	|||	|||	|||	|||	|||
Vibrio brevis	−	−	|||	|||	|||	|||	|||	|||	|||
Vibrio fulvus	−	−	−	−	|||	|||	|||	|||	|||
Vibrio auranriacus	−	−	||	−	|||	|||	|||	|||	|||
Vibrio marinotypicus	−	−	|||	|||	|||	|||	|||	|||	|||
Vibrio euryhalis	−	−	|||	|||	|||	|||	|||	|||	|||
Vibrio marinoflavescens	−	−	|||	|||	|||	|||	|||	|||	|||
Vibrio xanthus	−	−	||	||	|||	|||	|||	|||	|||
Cytophaga haloflava	−	−	−	−	|||	|||	|||	|||	|||
Cytophaga haloflava var. *nonreductans*	−	−	−	−	|||	|||	|||	|||	|||
Cytophaga rosea	−	−	−	−	−	−	|||	|||	|||

− = no growth; ||| = maximum growth.

Therefore, it is probably effective for keeping fishing nets for a long time in a good state to disinfect the nets immediately after fishing by treating them with a suitable disinfectant such as mercuric chloride, penta chlor phenol, or 5-nitro-2-furfuryl semicarbazone.

Such treatment of cotton fishing nets was recently carried out and found to be effective for their preservation.[84)]

Table 32. Disinfection of the marine aerobic cellulose-decomposing bacteria with various concentrations of 5-nitro-2-furfuryl semicarbazone at 15°C.

Concentration of 5-nitro-2-furfuryl semicarbazone / Contact time in minutes / Organism	1 : 20,000*		1 : 100,000		1 : 1,000,000		0
	10	30	10	30	10	30	
Pseudomonas marinocellulosae	###	##	###	###	###	###	###
Vibrio aquamarinus	—	—	###	—	###	###	###
Vibrio purpureus	###	##	###	###	###	###	###
Vibrio purpureus var. *albus*	###	##	###	###	###	###	###
Vibrio gilvus	—	—	##	##	###	###	###
Vibrio albogilvus	###	###	###	###	###	###	###
Vibrio macerans	—	—	###	###	###	###	###
Vibrio marinoliquefaciens	###	###	###	###	###	###	###
Vibrio simplex	—	—	—	—	###	###	###
Vibrio ferrugineus	###	##	###	###	###	###	###
Vibrio brevis	###	###	###	###	###	###	###
Vibrio fulvus	###	##	###	###	###	###	###
Vibrio aurantiacus	—	—	###	##	###	###	###
Vibrio marinotypicus	###	###	###	###	###	###	###
Vibrio euryhalis	###	###	###	###	###	###	###
Vibrio marinoflavescens	###	###	###	###	###	###	###
Vibrio xanthus	—	—	—	—	###	###	###
Cytophaga haloflava	—	—	###	—	###	###	###
Cytophaga haloflava var. *nonreductans*	—	—	###	###	###	###	###
Cytophaga rosea	—	—	###	###	###	###	###

— = no growth; ### = maximum growth.

* 5-nitro-2-furfuryl semicarbazone was not soluble to water at the concentration of more than 1 : 20,000 under this condition.

As disinfectants for the above mentioned purposes mercuric chloride, penta chlor phenol, or 5-nitro-2-furfuryl semicarbazone appear to be suitable, and the common net-preservatives such as copper sulfate and cutch appear to be unsuitable.

Table 33. Disinfection of the marine aerobic cellulose-decomposing bacteria with various concentrations of cutch at 15°C.

Concentration of cutch / Contact time in minutes / Organism	1 : 100		1 : 1,000		1 : 10,000		0
	10	30	10	30	10	30	
Pseudomonas marinocellulosae	++	++	++++	++++	++++	++++	++++
Vibrio aquamarinus	+	−	++++	++++	++++	++++	++++
Vibrio purpureus	−	−	++++	++++	++++	++++	++++
Vibrio purpureus var. *albus*	−	−	++++	++++	++++	++++	++++
Vibrio gilvus	−	−	++++	++++	++++	++++	++++
Vibrio albogilvus	−	−	++++	++++	++++	++++	++++
Vibrio macerans	−	−	++++	++++	++++	++++	++++
Vibrio marinoliquefaciens	++++	++++	++++	++++	++++	++++	++++
Vibrio simplex	++	++++	++++	++++	++++	++++	++++
Vibrio ferrugineus	−	−	++++	++++	++++	++++	++++
Vibrio brevis	−	−	++++	++++	++++	++++	++++
Vibrio fulvus	++++	++++	++++	++++	++++	++++	++++
Vibrio aurantiacus	−	−	++++	++++	++++	++++	++++
Vibrio marinotypicus	++++	++++	++++	++++	++++	++++	++++
Vibrio euryhalis	++++	++++	++++	++++	++++	++++	++++
Vibrio marinoflavescens	++++	++++	++++	++++	++++	++++	++++
Vibrio xanthus	−	−	++++	++++	++++	++++	++++
Cytophaga haloflava	++++	++++	++++	++++	++++	++++	++++
Cytophaga haloflava var. *nonreductans*	++++	++	++++	++++	++++	++++	++++
Cytophaga rosea	++++	++++	++++	++++	++++	++++	++++

− = no growth ; ++++ = maximum growth.

X. Prevention of microbiological damage of fishing nets by chemical modification and by toxic disinfectants

The efficacy of several rot proofing treatments for preventing the microbiological breakdown of cellulose fabrics of fishing nets during immersion in the sea was studied in the present chapter.

1. Prevention of microbiological damage of cellulose fabrics of fishing nets by chemical modification.

As shown in chapter VIII, cellulose acetates exhibited, in vitro, a higher resistance to action of the individual of the marine aerobic cellulose-decomposing bacteria than native cellulose. In order to confirm this resistance under natural environments, cellulose fabrics with different degrees of acetylation were exposed to the sea and then studied. The method employed was as follows:

Samples of cellulose acetate with various degrees of substitution were prepared from the acetylation of purified yarns of ramie (60 counts: single yarn).

The acetyl content of each sample was 0, 7.7, 26.6, 31.2, 37.0, 46.5, 53.7, and 61.5%, respectively. These acetate samples were immersed in sea water at the station near the floating pier of Kyoto University in Maizuru Bay from July to August, 1955. After immersion for the desired periods, the wet tensile strength of the yarns was measured by the usual method.

The results obtained were given in Table 34. This table shows that acetylated ramie yarns with acetyl contents varying from 26.6 to 61.5% were completely resistant to microbiological attack in the sea throughout the period of immersion.

Table 34. Per cent retention in tensile strength of aetylated yarns immersed in sea water at the station near the floating pier of Kyoto University in Maizuru Bay, from July to August, 1955 (temperature of the sea water: 25.1–30.0°C).

Immersion time in days \ % Acetyl content of fabric	0.0	7.7	26.6	31.2	37.0	46.5	53.7	61.5
0	100	100	100	100	100	100	100	100
10	89	86	101	88	95	100	107	90
20	57	85	99	78	96	94	90	99
30	40	77	106	98	94	102	95	112
41	32	47	106	104	102	90	102	105
51	21	33	107	98	104	102	105	111

The yarns with 7.7% acetyl content lasted considerably longer than the untreated yarns, but they were unable to endure more than 41 days. This result suggests that as long as there is at least one substituent on every anhydroglucose unit, complete resistance is obtained.

From the above mentioned fact, it is considered that fishing nets made of properly acetylated yarns are far more durable in the sea than those made of untreated yarns.

The only disadvantage connected with the use of acetylated yarns for fishing nets seems to lie in a slight weakness in tensile strength (Table 35). But, it appears to be possible that this obstacle can be circumvented by the improvement of the

acetylation process.

As stated in chapter VIII, it appears as if the nature of the substituent itself is of only secondary importance as to the microbiological susceptibility of cellulose derivatives. Therefore, when cellulose derivatives which are more superior in the mechanical properties than acetylated cellulose were found, such derivatives would be more suitable as the materials for fishing nets than acetylated cellulose.

Table 35. Initial tensile strength of acetylated and untreated ramie yarns (60 counts : single yarn).

% Acetyl content	Wet tensile strength (g)
0	833
7.7	823
26.6	763
31.2	791
37.0	785
46.5	799
53.7	775
61.5	657

2. Prevention of microbiological damage of cellulose fabrics of fishing nets by toxic disinfectants.

Disinfectants such as $HgCl_2$, penta chlor phenol, and 5-nitro-2-furfuryl semicarbazone which were found, in chapter IX, to be highly toxic to the individual of marine aerobic cellulose-decomposing bacteria were applied to ramie yarns exposed to sea water.

The relative efficacy of these disinfectants was studied by the following procedure: Samples of ramie yarn (60 counts : single yarn) were immersed in sea water at the station near the floating pier of Kyoto University in Maizuru Bay from July to August, 1955.

Table 36. Per cent retention in tensile strength of ramie yarns intermittently treated with toxic disinfectants during immersion in sea water at the station near the floating pier of Kyoto University in Maizuru Bay, from July to August, 1955 (temperature of the sea water : 25.1–30.0°C).

Immersion time in days \ Disinfectant	$HgCl_2$	P.C.P.	None (control)
0	100	100	100
10	89	89	89
20	80	97	57
30	64	85	40
41	37	39	32
51	33	33	21

During the period of immersion these samples were treated with $HgCl_2$ (1 : 10,000) or penta chlor phenol (1 : 2,000) every 10 days for 2 hours respectively.

After the immersion for desired periods, the wet tensile strength of the yarns was measured by the usual method.

The results obtained were given in Table 36.

This table shows that ramie yarns intermittently treated with $HgCl_2$ or penta chlor phenol lasted appreciably longer than the untreated yarns in sea water. When treated with these disinfectants the duration time of the ramie yarns was prolonged by about 10 days in sea water. The durability of the treated yarns, however, was far less than the yarns which had been acetylated to 26.6 to 61.5% in their acetyl content. From the above mentioned facts, it seems that the treatments by chemical modification are far superior to those by toxic disinfcetants in order to prevent microbiological damage of fishing nets during immersion in sea water.

Summary and Conclusion

Studies were primarily made of marine cellulose-decomposing bacteria in Maizuru Bay and in Hiroshima Bay to determine identity of the species involved and relative abundance under various ecological conditions. Additionally, some of the environmental factors influencing the activity of marine aerobic cellulose-decomposing bacteria, the important physiological activities of these bacteria, the behaviors of these organisms on cellulose and cellulose derivatives, and the disinfecting effects of several chemical agents upon these bacteria were investigated.

As a trial for application, prevention of microbiological damage of fishing nets by chemical modification and by toxic disinfectants was also attempted.

Conclusions obtained from the experimental results may be summarized as follows :

1. It was ascertained, using various kinds of media suitable for the growth of marine cellulose-decomposing bacteria, that aerobic cellulose-decomposing bacteria were widely distributed in sea water and in bottom deposit and that anaerobic cellulose-decomposing bacteria existed not at all in sea water and only sparsely in bottom deposit.

Therefore, it may be concluded from these results that the deterioration of fishing nets made of cellulose fibers, in sea water, is primarily caused by the activities of aerobic cellulose-decomposing bacteria.

2. Very superior media for the purposes of selective enrichment and enumeration of marine aerobic cellulose-decomposing bacteria were found by the present author, and 252 cultures of marine aerobic cellulose-decomposing bacteria were purely isolated, using these media.

All of the organisms isolated were classified and identified into 3 genera-18 species and 2 varieties, as follows :

Pseudomonas marinocellulosae, *Vibrio aquamarinus*, *Vibrio purpureus*, *Vibrio*

purpureus var. *albus*, *Vibrio gilvus*, *Vibrio albogilvus*, *Vibrio macerans*, *Vibrio marinoliquefaciens*, *Vibrio simplex*, *Vibrio ferrugineus*, *Vibrio brevis*, *Vibrio fulvus*, *Vibrio aurantiacus*, *Vibrio marinotypicus*, *Vibrio euryhalis*, *Vibrio marinoflavescens*, *Vibrio xanthus*, *Cytophaga haloflava*, *Cytophaga haloflava* var. *nonreductans*, and *Cytophaga rosea*.

Of these, *Pseudomonas marinocellulosae*, *Vibrio aquamarinus*, *Vibrio gilvus*, *Vibrio albogilvus*, *Vibrio macerans*, *Vibrio marinoliquefaciens*, *Vibrio simplex*, *Vibrio ferrugineus*, *Vibrio brevis*, *Vibrio fulvus*, *Vibrio aurantiacus*, *Vibrio marinotypicus*, *Vibrio euryhalis*, and *Vibrio xanthus* were proposed as new species.

3. It was found from the population counts that there were no definite seasonal cycles in the total abundance of aerobic cellulose-decomposing bacteria in sea water as well as in marine muds, but somewhat in the abundance of certain species of them in sea water. For example, *Vibrio purpureus*, *Vibrio purpureus* var. *albus*, and *Vibrio aquamarinus* predominated in summer, and *Vibrio marinoflavescens* and *Vibrio fulvus* in winter. The former group could grow at temperatures ranging from 20 to 35°C, and decomposed cellulose most rapidly at 30°C. The latter group, on the other hand, could not grow at temperatures higher than 30°C and grew most rapidly at 20–25°C. Therefore, the seasonal variations in the kinds of cellulose-decomposing bacteria found in sea water were probably attributable to the optimum growth temperatures of these organisms.

It seems that during summer months the deterioration of cotton fishing nets immersed in sea water is primarily caused by *Vibrio purpureus* group, but during winter months is mainly caused by *Vibrio marinoflavescens* group.

Since cellulose-decomposing activity of *Vibrio purpureus* group at 25–30°C was much greater than that of *Vibrio marinoflavescens* group at 15–25°C, seasonal fluctuation of the deterioration rate of cotton fishing nets exposed to the sea may partly be attributable to the seasonal variation in the kinds of cellulose-decomposing organisms in sea water.

4. It was found that cellulose-decomposing bacteria belonging to genus *Cytophaga* constituted from 0 to 33.3% of the total number of aerobic cellulose-decomposing bacteria present in sea water and in marine mud, and the abundance of these bacteria in sea water seemed to be closely related with that of planktons. It was also found that on the cotton fishing nets submerged in sea water cytophagas constituted from 22.2 to 40.5% of the total number of aerobic cellulose-decomposing bacteria, and that this percentage was more than twice as many as that found in the sea water at the same location.

This result indicates that cytophagas grew exclusively or preferentially on solid surfaces.

5. From the results of the observations on vertical and geographical distributions

of aerobic cellulose-decomposing bacteria in the sea, it was found that the population of these bacteria was always larger in bottom deposits than in overlying water, in which marked stratification of the organisms did not exist.

In some cases, it was observed that the abundance of aerobic cellulose-decomposing bacteria in bottom mud decreased with the distance from the shore. It seems well to consider, however, that this was caused by the quantity of the available organic matter present in the mud rather than by the distance from the shore or the depth of the overlying water, because this phenomenon was recognized only in those coastal regions of the sea receiving a large amount of land drainage, but hardly in the regions receiving only a little amount of land drainage.

Small abundance of aerobic cellulose-decomposing bacteria was found in mud, in spite of the presence of a large amount of organic matter, containing a large amount of H_2S.

6. By observing the changes in flora of cellulose-decomposing bacteria occurring on cotton fishing nets immersed in sea water, it was found that the deterioration of nets immersed in sea water was caused mainly by aerobic cellulose-decomposing bacteria belonging to *Eubacteriales* and partly by those belonging to *Cytophaga*.

It was found that a large number of cells of marine aerobic cellulose-decomposing bacteria grown on the cotton fishing nets immersed in the sea survived or developed for a considerably long period of storage under dry as well as wet conditions, and that under such conditions the surviving period of the cytophagas was much longer than that of the true bacteria.

It seems, therefore, that as regards the deterioration of nets stored after fishing or immersed again in sea water the cytophagas play a more important role than the true bacteria, although as regards the deterioration of nets newly immersed in sea water the true bacteria play a more important role than the cytophagas.

7. A few marine aerobic cellulose-decomposing bacteria were quickly injured at 40°C. However, the majority of them resisted 40°C for 40 minutes, but died if kept at 55°C for 5 minutes.

All of the marine aerobic cellulose-decomposing bacteria tested grew well in the pH ranging from 6.0 to 9.0 (most alkaline tested), but did not below the pH 5.0.

All of the marine aerobic cellulose-decomposing bacteria developed rapidly and luxuriantly on the media which were prepared with natural sea water or with a 3% solution of NaCl, but not on the media prepared with fresh water or with a 10% solution of NaCl. Only a few of them developed on the media prepared with the solutions of 1.0 and 5.0% NaCl. These facts suggest that all of the bacteria examined were specifically marine.

8. Marine aerobic cellulose-decomposing bacteria belonging to genera *Vibrio* and *Pseudomonas* could utilize more various organic compounds as a carbon source

than those belonging to genus *Cytophaga*, and most of the latter could not utilize a variety of organic compounds excepting only a specialized few compounds such as cellulose, cellobiose, and glucose as the sources of energy.

Among the organic compounds tested, only cellulose, cellobiose, and glucose were utilized by all species of the marine aerobic cellulose-decomposing bacteria as a source of energy. More than 60% of the isolated species utilized starch, sucrose, and galactose; about 50% of them utilized trehalose, lactose, maltose, mannose, fructose, arabinose, and xylose; 20–35% of them utilized inositol, mannitol, glycerin, raffinose, salicin, Ca-lactate, Ca-gluconate, ane Na-alginate; less than 20% of them utilized erythritol, agar, inulin, and rhamnose. None of the species employed utilized dulcitol.

9. Most of the marine aerobic cellulose-decomposing bacteria were evenly endowed with the ability to utilize a variety of nitrogen compounds. With the exception of $NaNO_2$, all the compounds tested were utilized by more than 80% of the organisms isolated. $NaNO_2$ was utilized by only two species, *Vibrio macerans* and *Cytophaga haloflava*.

Of the bacteria isolated, only one species *viz.*, *Vibrio simplex* was not endowed with the ability to utilize organic nitrogen compound.

10. When a filter paper was attacked by the marine aerobic cellulose-decomposing bacteria, its external appearance was changed. In this change, there were two different types, *viz.*, "weathering" and "liquefying". The "weathering" was caused by the bacteria, *Pseudomonas marinocellulosae*, *Vibrio purpureus*, *Vibrio purpureus* var. *albus*, *Vibrio aquamarinus*, *Vibrio gilvus*, *Vibrio albogilvus*, *Vibrio macerans*, and *Vibrio marinoliquefaciens*, which predominated in summer (preferring relatively high temperature for growth). In the cultures of these species, the paper was rapidly but incompletely destroyed and it became pulpy and macerated, so that when shaken with water it broke up at once into a large number of small fibre particles. While such a mode of destruction was a characteristic of the aforementioned species, it never occurred in cultures of *Vibrio* species predominating in winter and of *Cytophaga* species. The "liquefying" was caused by the *Vibrio* species predominating in winter and by *Cytophaga* species; the substrate was somewhat gradually, but completely destroyed, so that it became translucent at the air-water interface.

The development of the marine aerobic cellulose-decomposing bacteria on the cellulose agar plates was not always accompanied by the formation of enzymatic fields of cellulose liquefaction around the growth. The enzymatic zones developing around the colonies on the cellulose agar plates varied widely in appearance with the species.

On cellulose agar plates, the broad clear zones of complete cellulose dissolution

occurred around the colonies of the above mentioned vibrios predominating in winter, but did not around those of *Cytophaga* species, although clear fields occurred only beneath their colonies (in the immediate neighborhood of the organisms). In the cases of the vibrios predominating in summer, the clear fields of cellulose dissolution did not occur both around and beneath the colonies.

And also, it was found, from the experiments on the chemical changes (which were estimated by the copper number, the index (R), and the weight of the residual cellulose) of cellulose attacked by marine aerobic cellulose-decomposing bacteria, that by the vibrios predominating in summer cellulose was markedly denaturalized but hardly liquefied, e. g., the copper number of the attacked cellulose increased remarkably but the weight loss was very little, and that on the other hand, by the vibrios predominating in winter and by all of the cytophagas cellulose was liquefied but hardly denaturalized, e. g., the weight loss of the attacked cellulose was considerable, but the increase in the copper number was hardly recognizable.

That is to say, the visual observations on the mode of cellulose decomposition were clearly in accord with the results obtained from the chemical experiments.

The cellulose deterioration of "weathering" type was generally larger in velocity than that of "liquefying" type. The rapid deterioration of cotton fishing nets submerged in sea water in summer months seems to be mainly attributable to the fact that the microorganisms causing the cellulose decomposition of "weathering" type are predominated in that season.

11. Cellulose acetate with acetyl contents varying from 26.6 to 61.5% was totally resistant to attack of the marine aerobic cellulose-decomposing bacteria. On the cellulose acetate with acetyl content of 7.7%, however, about a half of organisms isolated grew as well as on the unacetylated cellulose.

Therefore, it may be suggested from the above results that cellulose acetate, with at least one acetyl group on every anhydroglucose unit in its structure, is completely resistant to attack of the marine aerobic cellulose decomposing bacteria, because in the cellulose acetate with acetyl content varying from 26.6 to 61.5% there were more than one substituent on every anhydroglucose unit, but in the samples with acetyl content of 7.7% there was at the most one substituent on every anhydroglucose unit.

It seems, therefore, that fishing nets made of cellulose acetate, of which the acetyl content is more than 26.6%, will hardly be rotted by the bacteria.

12. Among several compounds tested as disinfectants, mercuric chloride showed the highest potency in respect to the disinfecting effect upon the marine aerobic cellulose-decomposing bacteria. This compound perfectly disinfected such bacteria at as low a concentration as 1 : 10,000, and markedly injured them in a concentration of 1 : 100,000.

Copper sulfate, phenol, penta chlor phenol, and 5-nitro-2-furfuryl semicarbazone were more or less toxic to the marine aerobic cellulose-decomposing bacteria in concentrations lower than one per cent. However, cutch which has been widely used for the preservative of fishing nets since olden times was hardly toxic to these bacteria at as high a concentration as one per cent.

Although the fact that a compound is toxic when tested in test tubes is no definite guarantee of its effectiveness as a preservative for cotton fishing nets during immersion in the sea, it shows that such a compound is effective as a preservative for cotton nets during storage after fishing.

13. Chemical modification and disinfectant treatment which were thought, from the results obtained from the experiments carried out in vitro, to be effective for preventing microbiological damage of fishing nets were applied to ramie yarns immersed in the sea.

By observing the resistance of yarns of acetylated cellulose with different degrees of acetylation it was found that the yarns which had been acetylated to an acetyl content of 26.6 to 61.5% were completely resistant to microbiological attack in the sea, and that the yarns with 7.7% acetyl content lasted considerably longer than the untreated yarns, but they were unable to endure more than 41 days. These results were in accord with those obtained from the experiments carried out in vitro.

From the results of the experiment concerning the efficacy of disinfectant treatment for preventing the microbiological breakdown of cellulose fabrics of ramie yarns during immersion in sea water, it was found that ramie yarns intermittently treated with $HgCl_2$ or penta chlor phenol lasted about 10 days longer than the untreated yarns in sea water. The durability of the treated yarns, however, was far less than the yarns which had been acetylated to 26.6 to 61.5% in their acetyl content.

From the above mentioned facts, it may be concluded that the treatments by chemical modifications are far superior to those by toxic disinfectants in order to prevent microbiological damage of fishing nets during immersion in sea water.

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Appendix

A List of previously described species

Bacterium A Rubentschik[22]

Cells straight; 0.7 to 0.8 by 1.7 to 2.1 microns, occurring singly and in pairs. Actively motile. Gram-positive. Nonsporeforming.
Agar slant: Grayish.
Broth: Turbid with thin pellicle and sediment.
Gelatin: Not liquefied.
Milk: No change.
H_2S not produced from albuminous materials.
Ammonia not produced from albuminous materials.
Indol not formed from albuminous materials.
No pigment produced on cellulose in van Iterson's medium.
Salinity range: Good growth in 0 through 5.0%; maximum, 15.0%.
Aerobic.
Isolated from Kujalnizk-Liman (near Odessa).

Bacterium B Rubentschik

Cells straight; 0.5 to 0.7 by 1.0 to 1.2 microns, occurring singly and in pairs. Large cells of 15 microns long occur in old cultures. Actively motile. Gram positive. Nonsporeforming.
Agar slant: Grayish.
Broth: Turbid.
Gelatin: Slowly liquefied.
Milk: Peptonized.
H_2S not produced from albuminous materials.
Ammonia not produced from albuminous materials.
Indol not formed from albuminous materials.
No pigment produced on cellulose in van Iterson's medium.
Salinity range: Growth in 0 to 5.0%; Optimum, 0%.
Aerobic.
Isolated from Kujalnizk-Liman (near Odessa).

Vibrio agarliquefaciens (Gray and Chalmers) Bergey et al. (*Microspira agar-liquefaciens* Gray and Chalmers)[24][31]

Cells (marine strains) 0.5 to 1.5 by 1.0 to 10 microns, the larger cells apparently pleomorphic individuals. Many of the longer rods variously curved and bent; many average-sized rods slightly curved in young cultures. Single or dividing

pairs. Coccoid cells present. Encapsulated, nonsporeforming, Gram-negative; motile with a single polar flagellum.

Agar colonies: White to cream-colored, thin, deeply sunken in agar, agar liquefied. Walls of digested pit in agar often become perpendicular; gelase field wide with sharp margin; colonies 4.0 mm. in diameter had gelase field 14 mm. in diameter on agar containing 0.1% peptone.

Agar slant: Deep groove quickly formed followed by liquefaction of agar.

Broth: Turbid, pellicle thin or absent.

Gelatin: Not liquefied.

Milk: No change.

Indol not produced.

Nitrite produced from nitrate.

Ammonia, nitrite, and nitrate serve as sources of nitrogen.

Ammonia produced from urea; not detected from any other substance.

Hydrogen sulfide is produced.

Acetyl-methyl-carbinol not produced.

Starch hydrolyzed.

Acid from arabinose, xylose, fructose, galactose, glucose, mannose, cellobiose, lactose, maltose, and salicin.

Utilized without acid production: Sorbitol (slowly).

Not utilized: Rhamnose, sucrose, raffinose, inulin, ethyl alcohol, ethylene glycol, glycerol, dulcitol, inositol, and mannitol.

Amino acids utilized as source of both N and C: D-arginine (slowly).

Amino acids utilized as a nitrogen source: Aspartic acid, cystine, glutamic acid, L-proline, L-methionine, DL-phenyl alanine, DL-serine, and DL-valine.

Amino acids not utilized: DL-alanine, creatine, glycine, L-leucine, and tyrosine.

Other organic acids utilized: Of those tested, none.

Other organic acids not utilized: Acetic, n-butyric, citric, gluconic, lactic, maleic, malic, malonic, mucic, oxalic, propionic, succinic, and tartaric.

Water-soluble pigment not produced from tyrosine.

Cellulose, chitin, and alginic acid are utilized.

Seaweed gels: *Chondrus*, *Hypnea*, *Gracilaria* sp. rapidly digested, often liquefied; *Gymnogongrus* gel rapidly liquefied; *Agardhiella* and *Eucheuma* gels apparently not attacked.

Catalase positive.

Salinity range: No growth in fresh water media; fair growth in 0.5% sea salt media; good growth in 1.0 through 6.0%. Optimum growth in 2.0 through 5.0%.

Aerobic.

Optimum temperature 25°C.; range, 15°C through 35°C.

Source: Beach sand near laboratory pier, Beaufort, N. C. (Originally described from soil, Northhampstead, England).
Habitat: Soil and the sea.

Vibrio fuscus STANIER[29]

Cells small, slightly curved rods, single, sometimes in short chains; 0.7 by 1.5 to 5.0 microns. Motile, polar flagella. Not encapsulated, nonsporeforming, Gram-negative.
Agar colonies: Circular, smooth, glistening, translucent, entire, pale yellow at first, later bright yellow, then brownish; sharply sunken in agar; gelase field narrow, sharply defined. Agar softened, sometimes liquefied.
Agar slant: Filiform, smooth, glistening, translucent, somewhat sunken. Yellow water-soluble pigment produced. Old colonies become brownish.
Broth: Turbid with granular sediment and yellow pellicle.
Gelatin: Liquefied, stratiform.
Indol not produced.
Nitrite produced from nitrate.
Urease negative.
Hydrogen sulfide not produced.
Starch, chitin, and alginic acid not attacked.
Cellulose slowly digested.
Utilizes xylose, galactose, glucose, cellobiose, lactose, maltose, and sucrose.
Arabinose not utilized.
Catalase positive.
Optimum temperature 20° to 25°C.
Aerobic.
Habitat: Sea water.

Pseudomonas iridescens STANIER[29][31]

Cells long, slender, straight, single rods; nonmotile; 0.3 to 0.5 by 2.5 to 7.5 microns; majority 3.0 to 4.0 microns long. Nonsporeforming, not encapsulated, Gram-negative.
Agar colonies: Spreading, thin, translucent, orange-yellow, developing greenish-gold iridescence. Margin irregular. Gelase field very wide and sharply delimited. Colonies rather evenly sunken in agar, i.e., flat rather than concave; agar digested and softened, not liquefied.
Agar slant: Thin, yellow streak becoming iridescent and spreading over entire slant surface.
Broth: Turbid; thin pellicle with yellow granules.

Gelatin: Liquefied, napiform or infundibuliform, then stratiform.

Milk: Slightly more alkaline, slowly peptonized; no growth with some strains.

Indol not produced: Slowly utilized.

Nitrite not produced from nitrate.

Ammonia and nitrate may serve as nitrogen source with some strains, but growth is less abundant than with peptone; some strains (Pacific coast) require organic nitrogen; nitrite is slowly utilized.

Urea utilized by some strains, usually without ammonia production.

Hydrogen sulfide not produced.

Acetyl-methyl-carbinol not produced.

Starch slowly hydrolyzed.

Acid from xylose, fructose, galactose, glucose, mannose (slight), cellobiose, lactose, maltose, salicin, glycerol, and mannitol.

Utilized usually without acid production: Arabinose (slowly), sucrose, and inulin (slowly).

Not utilized: Rhamnose, raffinose, ethyl alcohol, ethylene glycol, dulcitol, inositol, and sorbitol.

Amino acids utilized as source of N and C: D-arginine (slowly), glutamic, and L-proline.

Amino acids utilized only as nitrogen source: DL-alanine, L-aspartic acid, L-leucine, tyrosine (slowly), and sometimes cystine, glycine, L-methionine, DL-serine, and DL-valine.

Amino acids not utilized: Creatine, and DL-phenylalanine.

Other organic acids utilized: Lactic and, sometimes, acetic and succinic.

Other organic acids not utilized: n-butyric, citric, gluconic, maleic, malic, malonic, mucic, oxalic, propionic, tartaric, and iso-valeric.

Alginic acid hydrolyzed by Atlantic coast strains but not by Pacific coast strains.

Cellulose hydrolyzed by Pacific coast strains, but not by Atlantic coast strains.

Chitin not attacked.

Seaweed gels: *Agardhiella*, *Eucheuma*, *Gymnogongrus* rapidly liquefied with acid production; *Chondrus*, *Hypnea*, and *Gracilaria* sp. digested but not liquefied.

Catalase positive (vigorously).

Salinity range: No growth in fresh water media; growth in 0.5 through 6.0% sea salt; Optimum 2.0 to 4.0%.

Aerobic.

Optimum temperature 25° to 30°C., range 15° to 40°C.

Source: Beach sand, Clearwater Bay at Dunedin, Florida, March, 1943; from *Gracilaria cornea*, Tempa Bay, Florida, July 1943. California coast by Stanier (1941).

Habitat: Probably widely distributed and common in the sea, especially in temperate regions.

Cytophaga krzemieniewskae STANIER[29)31)49)]

Morphology: Long, very flexible rods, 0.5–1.5 by 5.0–20.0 microns. Gram negative. Star-shaped aggregates are common in liquid media. Coccoid involution forms frequently occur in old cultures.

Sea-water peptone agar plate: Growth begins as a smooth, pale pink rapidly spreading swarm. After a few days, the older portion of the swarm assumes a warty appearance due to the accumulation of the cells in drop-like masses resembling immature fruiting bodies, but always containing normal vegetative cells. A diffusible brown to black pigment which masks the pink color of the swarm is produced after about a week. Agar is rapidly decomposed, and ultimately liquefaction becomes almost complete.

Sea-water gelatin stab: Liquefaction.

Growth in liquid media (sea-water peptone broth, sea-water glucose peptone broth) is turbid and silky with a pink sediment; the medium turns dark brown or black after 1–2 weeks.

Utilizable carbon sources: Xylose, glucose, galactose, lactose, maltose, cellobiose, cellulose, alginic acid, agar, and starch; also proteinaceous materials.

Utilizable nitrogen sources: Yeast extract and peptone are the only suitable ones known.

Weakly catalase positive.

Indol not formed.

Salt concentration range: 1.5–5.0 per cent.

Strictly aerobic.

Temperature range: 15–30°C. Optimum 22–25°C.

Source: Sea water.

Habitat: Probably decaying marine vegetation.

Cytophaga diffluens STANIER[29)31)49)]

Morphology: Flexible rods, 0.5–1.5 by 4.0–10.0 microns. Gram negative. In old cultures involution forms consisting of long twisted, thin threads are found. Star-shaped aggregates of cells occur in liquid media.

Sea-water peptone agar plate: Growth begins as a thin, pink, rapidly spreading swarm which covers the entire surface in a few days. The swarm gradually increases in thickness and develops an irregular, beaten-copper surface due to the liquefaction of the underlying agar. After 4–5 days the color becomes orange. Liquefaction of the agar is ultimately almost complete.

Sea-water gelatin stab: Rapid liquefaction.

Growth in liquid media is turbid, often with suspended floccules and a heavy pellicle.

Utilizable carbon sources: Xylose, glucose, galactose, lactose, maltose, cellobiose, cellulose, agar, and alginic acid; also proteinaceous materials.
Utilizable nitrogen sources: Yeast extract and peptone are the only suitable ones known.
Weakly catalase positive.
Indol not formed.
Salt concentration range: 1.5–5.0 per cent.
Strictly aerobic.
Temperature range: 15–30°C. Optimum 22–25°C.
Source: Sea water.
Habitat: Probably decaying marine vegetation.

Vibrio granii (LUNDESTAD) STANIER[29)30)31)]

Cells small, curved rods, single, occasional chains; 0.5 by 2.0 to 4.0 microns. Cells straight in old cultures. Motile, polar flagella. Encapsulated, nonsporeforming, Gram negative.
Agar colonies: Circular, smooth, glistening, entire, flat, of slimy consistency, sunken in agar. Agar rapidly digested, sometimes liquefied.
Agar slant: Smooth, glistening, filiform, bluish translucency; surface becomes wrinkled and membranous with some strains; agar liquefied with production of acid.
Broth: Heavy white pellicle, turbid. Turbidity may be delayed with some strains; broth becomes viscous, stringly.
Gelatin: Slow crateriform liquefaction.
Indol not produced.
Nitrite produced from nitrate.
Urease negative.
Hydrogen sulfide not produced.
Starch hydrolyzed by some strains only.
Cellulose hydrolyzed; chitin and alginic acid not attacked.
Utilizes arabinose, xylose, galactose, glucose, lactose, maltose, sucrose, and cellobiose.
Catalase positive.
Aerobic.
Optimum temperature: 20 to 25°C.
Habitat: Sea water.

Pseudomonas droebachense (LUNDESTAD) STANIER (*Flavobacterium droebachense* (LUNDESTAD) BERGEY et al.; *Bacterium droebachense* LUNDESTAD).[29)30)31)]

Cells rods, nonmotile, slender, straight, single, 0.3 to 0.4 by 2.0 to 10 microns. Chains or filaments produced by one strain. Nonsporeforming, not encapsulated,

Gram negative.

Agar colonies: Yellow, orange-yellow, or brownish-orange, raised with small prominence in center, margin irregular. Central portion of colony opaque, margin thin, translucent, spreading. Gelase field narrow, fading. Colonies form barely visible depressions in agar; later they become membranous, adherent. Not iridescent.

Agar slant: Filiform at first, orange-yellow, slowly spreading and becoming orange-brown, especially at basal portion. Growth becomes tough and stringy; not iridescent.

Broth: Moderate turbidity often clearing after two or three days. Pellicle thin, granular, yellow. Granules may adhere to sides of tube.

Gelatin slowly liquefied, crateriform to stratiform.

Milk: No growth.

Indol not produced; slowly utilized.

Nitrite produced from nitrate by most strains.

Ammonia, nitrite, or nitrate serve as nitrogen source with some strains; others require organic nitrogen source.

Ammonia produced from peptone, asparagin, and DL-valine.

Urea utilized as nitrogen source by most strains without production of ammonia.

Hydrogen sulfide not produced.

Acetyl-methyl-carbinol not produced.

Starch slowly hydrolyzed by most strains.

Acid from arabinose, xylose, rhamnose, fructose, galactose, glucose, mannose, cellobiose, lactose, maltose, sucrose, raffinose, inulin, and salicin.

Not utilized: Ethyl alcohol, ethylene glycol, glycerol, dulcitol, inositol, mannitol, and sorbitol.

Amino acids utilized as source of N and C: D-arginine and glutamic acid by some strains; L-proline by one strain.

Amino acids utilized only as nitrogen source: DL-alanine, L-aspartic acid, cystine, L-leucine, L-methionine, L-proline, and DL-valine.

In addition, one strain utilized DL-serine.

Amino acids not utilized: Glycine, creatine, DL-phenylalanine, DL-serine, and tyrosine.

Other organic acids utilized: One strain utilized acetic, lactic, and succinic.

Other organic acids not utilized: n-butyric, citric, gluconic, maleic,malic, malonic, mucic, oxalic, propionic, tartaric, and iso-valeric.

Water-soluble pigment formed from tyrosine after three or four weeks.

Cellulose, chitin, and alginic acid not attacked by Atlantic coast strains; cellulose and alginic acid hydrolyzed by Pacific coast strains.

Seaweed gels: *Hypnea*, *Chondrus*, *Agardhiella*, *Gracilaria* sp. slowly utilized.

Catalase positive (vigorous).

Salinity range: No growth in fresh water media; fair growth in 0.5% sea salt; good growth, 1.0 through 6.0%; optimum, 3.0 through 5.0%.

Aerobic.

Optimum temperature 25°C.; range, 5 to 35°C.

Source: Several strains from the alga, *Gracilaria blodgettii*, collected at Piney Point (Tampa Bay), Florida, April, 1943; one strain from sea water near laboratory, Beaufort, N. C., October, 1942. From water along California coast (STANIER, 1941).

Habitat: Common on algae, Gulf of Mexico and along lower Atlantic coast of the U. S. Probably widely distributed in the sea.

Vibrio notus HUMM[31]

Cells rods, slightly curved in young cultures, motile, single; 0.5 to 1.0 by 1.5 to 3.0 microns; at times pleomorphic but usually cells are fairly uniform. Nonspore-forming, not encapsulated, Gram-negative.

Agar-colonies: Pale yellow to white, sharply and deeply sunken in agar, forming a pit that may have vertical sides after a week or more; gelase field with sharply delimited margin.

Agar slant: Streak forms a groove and soon liquefies the agar with considerable acid production.

Broth: Clear to slightly turbid; pellicles usually not formed.

Gelatin: Not liquefied.

Milk: No change.

Indol not produced; not utilized.

Nitrite not produced from nitrate.

Ammonia, nitrite, or nitrate serve as source of nitrogen.

Ammonia from asparagin, nitrite and nitrate; no ammonia from peptone.

Peptone utilized as nitrogen source only; no growth unless suitable carbon source is supplied in addition. Nitrate is superior nitrogen source.

Hydrogen sulfide not produced.

Acetyl-methyl-carbinol not produced.

Starch vigorously hydrolyzed.

Acid from xylose, fructose, galactose, glucose, mannose, cellobiose, lactose, maltose, and salicin.

Utilized without acid production: Sorbitol (slowly).

Not utilized: Arabinose, rhamnose, sucrose, raffinose, inulin, ethyl alcohol, ethylene glycol, mannitol, dulcitol, and inositol.

Acid production from sugars not especially strong.

Amino acids utilized as sole source of N and C: None of those tested.

Amino acids utilized only as nitrogen source: D-arginine (poorly), aspartic acid

(poorly), L-leucine, glutamic acid, DL-serine, and tyrosine (poorly).
Amino acids not utilized: DL-alanine, creatine, cystine, glycine, L-methionine, DL-phenylalanine, L-proline, and DL-valine.
Other organic acids utilized: None of those tested.
Pigment not produced from tyrosine.
Cellulose, chitin, and alginic acid hydrolyzed.
Seaweed gels: *Eucheuma* and *Gracilaria* sp. rapidly liquefied; *Hypnea*, *Chondrus*, and *Agardhiella* digested.
Catalase positive.
Salinity range: No growth in fresh water media or in 0.5% sea salt media; poor growth in 1.0%; good growth in 1.5 through 6.0%, optimum 5.0 to 6.0%.
Aerobic.
Optimum temperature 25°C.
Source: Isolated from intertidal sand, Atlantic Beach, N. C., June, 1941. Isolated from water at end of laboratory pier, Beaufort, N. C., May and October, 1943.
Habitat: The sea.

Vibrio stanierii Humm[31]

Cells curved or slightly curved rods, motile, 0.4 to 0.7 by 2.5 to 5.0 microns; single or in pares, coccoid cells common, occasional chains.
Nonsporeforming, not encapsulated, Gram-negative.
Agar colonies: Pale yellow or cream-colored, deeply and sharply sunken in agar, the gelase field scarcely beyond the almost vertical walls of the pit; gelase field with sharp margin, especially on nonnutrient agar.
Agar slant: Pale yellow, sharply sunken streak; agar liquefied rapidly.
Broth: Turbid with thin, almost colorless pellicle.
Gelatin: Not liquefied.
Milk: Unchanged or slightly more alkaline; peptonization not observed.
Indol not produced; slowly utilized.
Nitrite not produced from nitrate.
Ammonia, nitrite, or nitrate serves as source of nitrogen.
Ammonia from peptone, asparagin, urea (abundant), nitrite, and nitrate.
Hydrogen sulfide produced.
Urea utilized rapidly with abundant ammonia production (dumbbell-shaped crystals among cells forming pellicle in urea broth).
Acetyl-methyl-carbinol not produced.
Starch slowly hydrolyzed.
Acid from arabinose, xylose, fructose, galactose, glucose, mannose, cellobiose, lactose, maltose, and salicin.

Utilized without acid production: None. (Acid production often weak and transitory).
Not utilized: Rhamnose, sucrose, raffinose, inulin, ethyl alcohol, ethylene glycol, glycerol, dulcitol, inositol, mannitol, and sorbitol.
Amino acids utilized as source of N and C: L-leucine (poorly), and glutamic acid.
Amino acids utilized only as nitrogen source: DL-alanine, D-arginine, aspartic acid, and L-proline.
Amino acids apparently not utilized: Creatine, cystine, glycine, L-methionine, DL-phenylalanine, DL-serine, tyrosine, and DL-valine.
Other organic acids utilized: Succinic (slowly).
Other organic acids not utilized: Acetic, n-butyric, citric, gluconic, lactic, maleic, malic, malonic, mucic, oxalic, propionic, tartaric, and iso-valeric.
Water-soluble pigment not produced from tyrosine.
Cellulose and alginic acid hydrolyzed.
Chitin apparently not attacked.
Seaweed gels: *Agardhiella*, *Eucheuma*, *Gymnogongrus* and *Gracilaria* sp. gels rapidly liquefied with acid production; *Hypnea* and *Chondrus* gels digested.
Catalase positive.
Salinity range: No growth in fresh water media; slight growth in 0.5% sea salt; moderate growth in 1.0 and 1.5%; optimum growth in 2.0 through 6.0%.
Aerobic.
Optimum temperature 25° to 30°C.
Source: isolated from the seaweed *Acanthophora spicifera* collected in Biscayne Bay, Miami, Florida, September, 1942.
Habitat: The sea.

Pseudomonas elongata HUMM[81]

Cells slender, motile, single and in chains or filaments of indefinite length in which the elements could not be discerned; 0.3 to 0.4 by 3.0 to 6.0 microns or more. Coccoid cells present. In older cultures, the cells are shorter. Nonsporeforming, encapsulated, Gram negative.
Agar colonies: Thin, translucent, gray-white, rather deeply sunken in agar, agar liquefied; yellowish-brown, water soluble pigment produced from peptone after one week or more. Gelase field wide with distinct margin. Colony 5 mm in diameter had gelase field 16 mm in diameter.
Agar slant: Streak rapidly spreading, usually covering entire surface of slant; grayish-white, thin, translucent, viscous, stringy; liquefied agar yellowish-brown in color.
Broth: Turbid with thin pellicle, or clear with heavy pellicle.
Gelatin: Slowly liquefied, crateriform or saccate to stratiform.

Yellowish-brown water-soluble pigment slowly produced.
Milk: Becomes more alkaline, slowly peptonized; litmus decolorized at bottom of tube.
Indol not produced; slowly utilized.
Nitrite not produced from nitrate.
Ammonia, nitrite or nitrate serves as source of nitrogen.
Ammonia from peptone and asparagin (yellow-brown water-soluble pigment also produced from asparagin).
Urea apparently not utilized.
Hydrogen sulfide is produced.
Acetyl-methyl-carbinol not produced.
Starch hydrolyzed.
Acid from arabinose, xylose, cellobiose, maltose, sucrose, and salicin.
Utilized without acid production: Fructose, galactose, glucose, mannose, and lactose.
Not utilized: Rhamnose, raffinose, inulin, ethyl alcohol, ethylene glycol, glycerol, mannitol, dulcitol, inositol, and sorbitol.
Amino acids utilized as source of nitrogen and carbon: D-arginine, glutamic acid, and L-leucine.
Amino acids utilized only as nitrogen source: DL-alanine, aspartic acid, cystine, glycine, L-proline (poorly), and tyrosine (poorly).
Amino acids not utilized: Creatine, L-methionine, DL-phenylalanine, DL-serine, and DL-valine.
Other organic acids utilized: Acetic, lactic, malic, propionic, and succinic.
Other organic acids not utilized: n-butyric, citric, gluconic, maleic, malic, malonic, mucic, oxalic, tartaric, and iso-valeric.
Water-soluble pigment not produced from tyrosine.
Cellulose, chitin, and alginic acid hydrolyzed.
Seaweed gels: *Eucheuma*, *Hypnea*, *Gracilaria* sp. gels liquefied; *Chondrus* gel digested; *Agardhiella* gel apparently not utilized.
Catalase positive.
Salinity range: No growth in fresh water media; poor growth in 0.5% sea salt; optimum growth in 2.0 and 3.0%; good growth in 1.0 through 6.0%.
Aerobic.
Optimum temperature 25 to 30°C.
Source: Isolated from intertidal sand, Atlantic Beach, N. C., October, 1941.
Habitat: Sea water and bottom sediments.

Cellvibrio sp. Hidaka[8]

Cells curved or slightly curved slender rods with rounded ends, 0.5 to 0.9 by 2.5

to 6.0 microns, single. Motile, polar mono flagellum.
Nonsporeforming, not encapsulated. Gram negative.
Cellulose agar colonies: Small, circular, smooth, flat, glistening, translucent, yellow, surrounded with enzymatic clear zones.
Cellulose agar slant: Smooth, glistening, flat, filiform, yellow, semi-opaque.
Agar slant: Smooth, glistening, flat, filiform, yellowish brown, semi-opaque.
Starch agar slant: Flat, echinulate, yellow, semi-opaque.
Growth on cellulose: In tubes of liquid mineral medium (prepared with sea water) with filter paper partly immersed, growth becomes apparent after 2–3 days (at 30°C) as a yellow area on the paper at the air-water interface. The liquid becomes turbid and semi-opaque. The filter paper at water level is completely decomposed after 6–7 days.
Broth: Turbid, pellicle formed.
Milk: No growth.
H_2S not produced.
Indol not produced.
NH_3 not formed from peptone in liquid medium (prepared with sea water) with filter paper partly immersed.
Nitrate not reduced.
Utilizable carbon sources: Cellulose, starch, mannan, Na-alginate, sucrose, maltose, and cellobiose.
Not utilized: glucose and lactose.
Utilizable nitrogen sources: $NaNO_3$, KNO_3, $(NH_4)_2SO_4$, NH_4Cl, peptone, asparagine, and casein.
Not utilized: $(NH_4)_2CO_3$, aspartic acid.
Temperature range for growth: 15–30°C.
Optimum temperature: 27°C.
pH range for growth: 5.8–9.0. Optimum pH: 6.5.
Salinity range for growth: 0.5–7.0%. Optimum salinity: 1.0%.
Aerobic.
Source: Intestinal contents of *Toredo navalis* sp.

Those studied by KOTANI[15] were not included because the descriptions were inadequate.

Explanation of Plates

Plate 1. Electron photo micrograph of *Pseudomonas marinocellulosae* nov. sp., 15,000 ×.
Plate 2. Electron photo micrograph of *Vibrio aquamarinus* nov. sp., 15,000 ×.
Plate 3. Electron photo micrograph of *Vibrio purpureus* KADOTA, 15,000 ×.
Plate 4. Electron photo micrograph of *Vibrio purpureus* var. *albus* KADOTA, 15.000 ×.

Plate 5. Electron photo micrograph of *Vibrio gilvus* nov. sp., 15,000 ×.
Plate 6. Electron photo micrograph of *Vibrio albogilvus* nov. sp., 15,000 ×.
Plate 7. Electron photo micrograph of *Vibrio macerans* nov. sp., 15,000 ×.
Plate 8. Electron photo micrograph of *Vibrio marinoliquefaciens* nov. sp., 15,000 ×.
Plate 9. Electron photo micrograph of *Vibrio simplex* nov. sp., 15,000 ×.
Plate 10. Electron photo micrograph of *Vibrio ferrugineus* nov. sp., 15,000 ×.
Plate 11. Electron photo micrograph of *Vibrio brevis* nov. sp., 15,000 ×.
Plate 12. Electron photo micrograph of *Vibrio fulvus* nov. sp., 15,000 ×.
Plate 13. Electron photo micrograph of *Vibrio aurantiacus* nov. sp., 15,000 ×.
Plate 14. Electron photo micrograph of *Vibrio marinotypicus* nov. sp., 15,000 ×.
Plate 15. Electron photo micrograph of *Vibrio euryhalis* nov. sp., 15,000 ×.
Plate 16. Electron photo micrograph of *Vibrio marinoflavescens* KADOTA, 15,000 ×.
Plate 17. Electron photo micrograph of *Vibrio xanthus* nov. sp., 15,000 ×.
Plate 18. Electron photo micrograph of *Cytophaga haloflava* KADOTA, 15,000 ×.
Plate 19. Electron photo micrograph of *Cytophaga haloflava* var. *nonreductans* KADOTA, 15,000 ×.
Plate 20. Electron photo micrograph of *Cytophaga rosea* KADOTA, 15,000 ×.
Plate 21. Colonies of *Vibrio aurantiacus* on cellulose agar plate.
Plate 22. Colonies of *Cytophaga haloflava* on cellulose agar plate.
Plate 23. Colonies of *Vibrio purpureus* on cellulose agar plate.

Plate 1

Plate 2

Plate 2

Plate 4

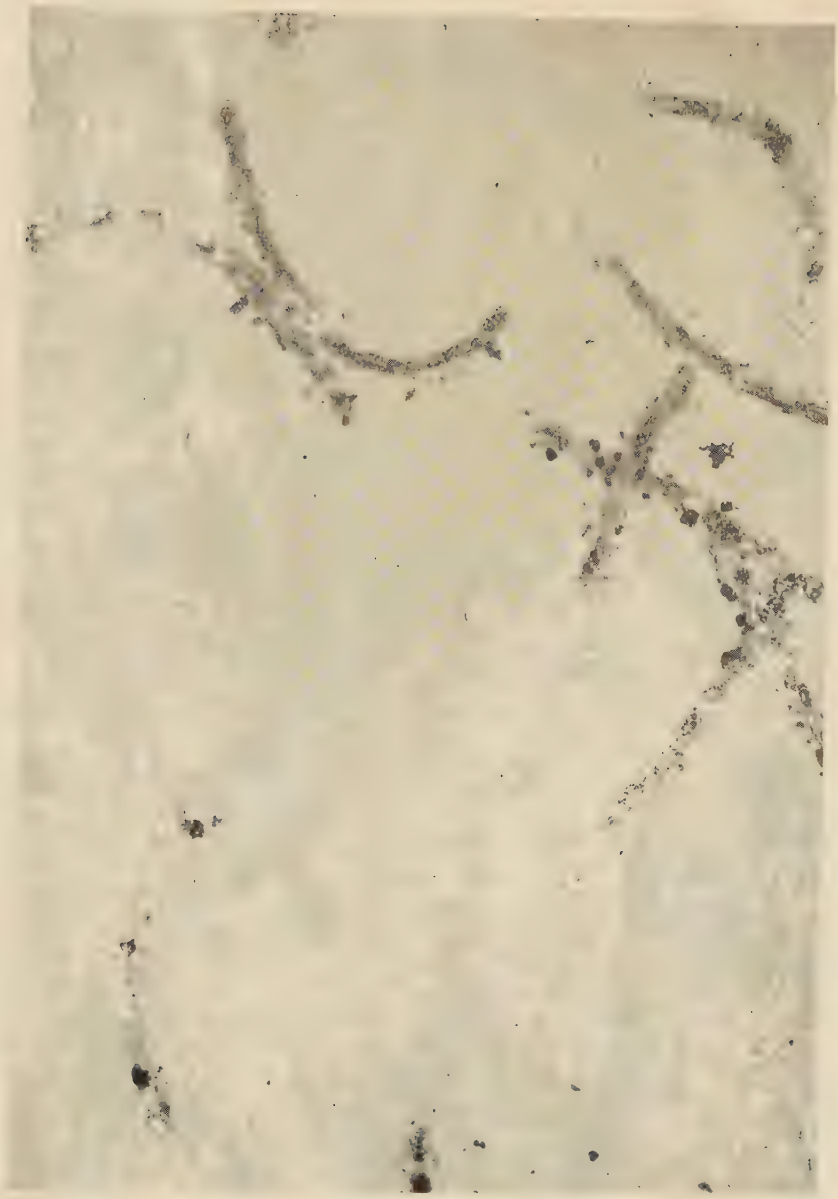

Plate 5

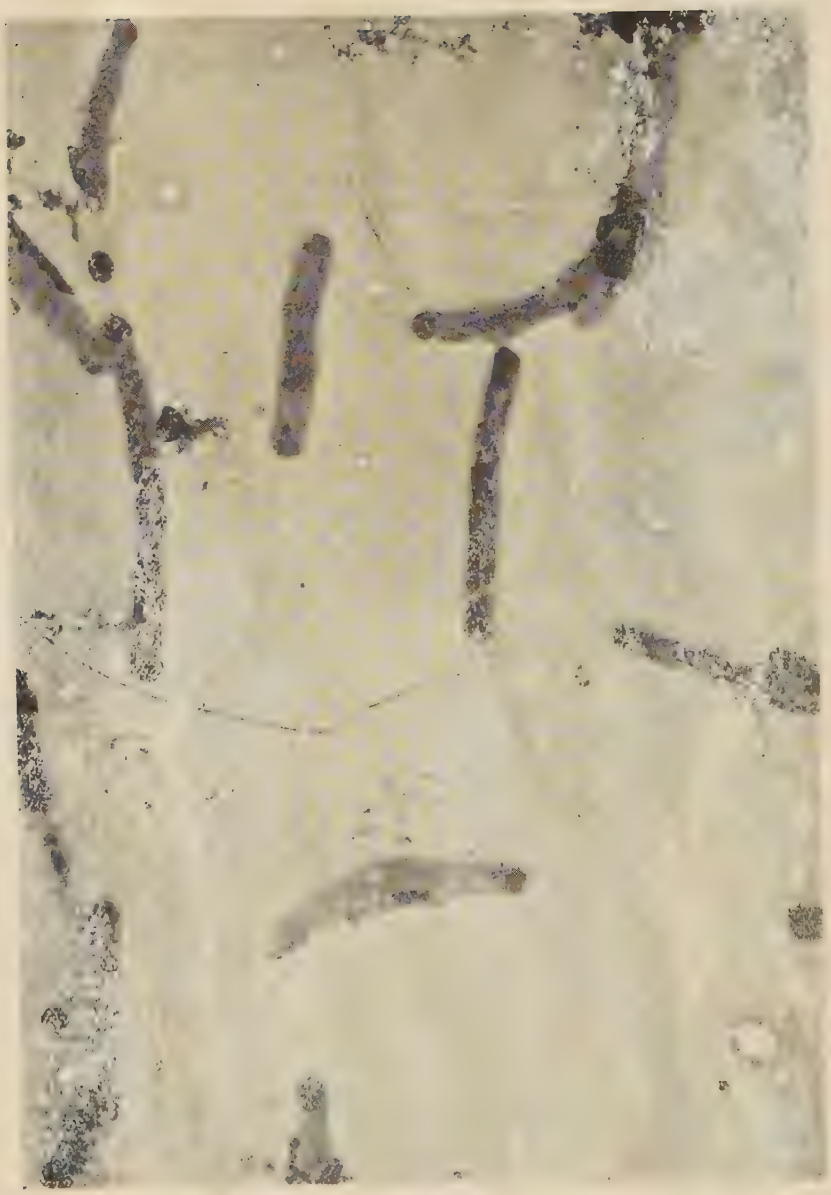

Plate 6

Plate 7

Plate 8

Plate 9

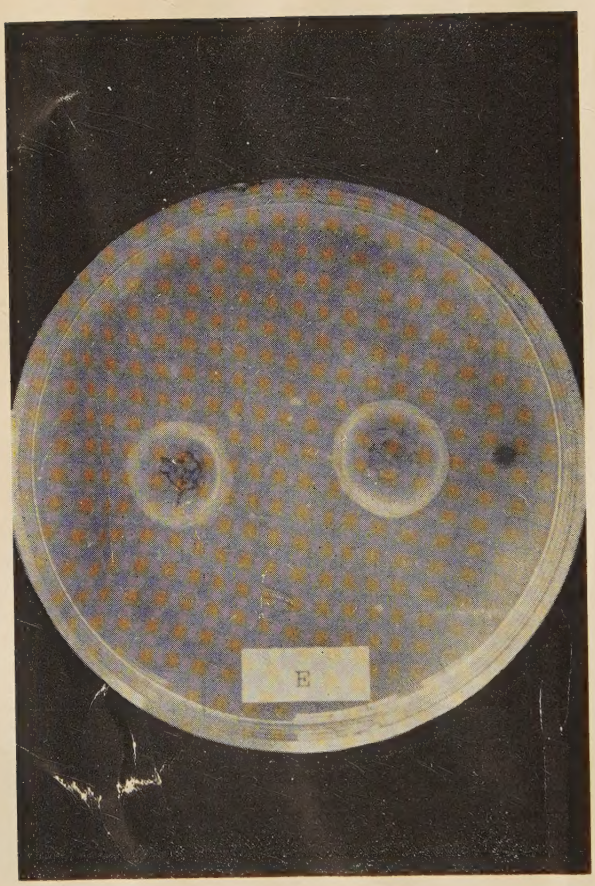

Plate 23

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